# ABSTRACT OF THESIS

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*Title of Thesis* CONFORMATIONAL STUDIES IN CARBOHYDRATE CHEMISTRY

I. The Rates of Formation of Some Hexoside 2,3-Epoxides

The secondary tosylates of some 4,6-O-ethylidene derivatives of compounds with the D-glucose configuration have been prepared, and their rates of cyclisation to 2,3-epoxides in alkaline solution have been measured. In excess sodium hydroxide solution, the cyclisation of the tosylates was found to exhibit kinetics first-order with respect to the concentration of the tosylate. The relative rates of reaction obtained from these experiments have been correlated with the structural and stereochemical features of the tosylates in terms of recent theories of reaction mechanism and conformational analysis. It is clear from the results that electronic as well as steric effects must be invoked to explain the differential reactivities found in these systems. Evidence relevant to the effect of conformational energy barriers ("passing interactions") on the rates of reaction is discussed.

II. The Aqueous Solvolysis of Glycoside 6-Tosylates

Methyl 6-O-tosyl-β-D-galactoside has been shown to react slowly in aqueous solution to produce methyl 3,6-anhydro-β-D-galactoside, toluene-p-sulphonic acid being released. Approximate rate-constants for the aqueous solvolyses of some 6-O-tosyl glycosides have been obtained, and the results critically compared with those for the corresponding reactions in alkali.

III. Some Experiments on the Alkaline Fragmentation of Methyl 2-O-tosyl-α-D-glucoside

Methyl 2-O-tosyl-α-D-glucoside has been found to undergo partial fragmentation on reaction with aqueous sodium hydroxide, enolic products being formed, as shown by the ultraviolet spectra in acidic and alkaline solution. Attempts to identify the products by preparing crystalline derivatives have so far proved unsuccessful.

*Use other side if necessary.*

# CONFORMATIONAL STUDIES IN CARBOHYDRATE CHEMISTRY

by

H. D. Mathewson

Thesis presented for the degree of Doctor of Philosophy

at the University of Edinburgh.

1963

## A C K N O W L E D G E M E N T S

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CONTENTS

# INTRODUCTION

The structures and stereochemical configurations of monosaccharides and their derivatives are well established, and the methods for their elucidation highly developed. With this knowledge behind them, carbohydrate chemists have been directing their attentions more and more towards a quantitative understanding of the reactions of sugars.

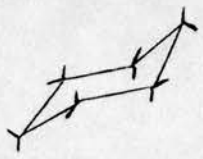
It is well known, for all chemical systems, that a complete understanding of reaction mechanism is only possible by consideration of two main factors: the so-called "electronic" and "steric" factors. The electronic factor generally requires knowledge of the situation and movement of electrons in the immediate environment of the reacting centres. This factor is fairly well understood in simple systems, [1,2] and the principles derived from the study of such systems can often be applied, at least in a qualitative manner, to the reacting portions of more complex molecules. While chemists have long acknowledged the importance of steric factors in understanding reactivities, it is only recently that such concepts have been formulated in anything like quantitative terms. The development of the principles of conformational analysis has played an important role in the theoretical interpretation of steric effects, and it is the application of these principles to carbohydrate systems which forms the basis of this work.

Conformational analysis, and its development over the last 10 years or so, have been well reviewed[3,4,5,6]. The principles of this complex subject were based largely on work in the field of cyclohexane chemistry, and were derived

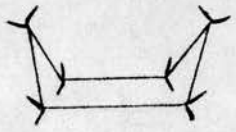
from theoretical interpretation of the physical and chemical properties of cyclohexane and its derivatives. The general conclusions are as follows:-

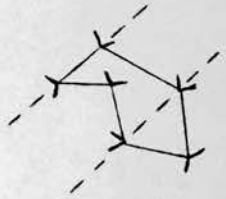
Molecular Shape

The cyclohexane molecule can exist in two geometric arrangements which do not distort the carbon tetrahedral bond angle.



Chair form (a)





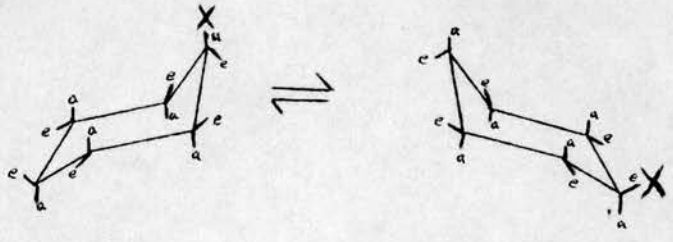
Boat and Skew forms (b)

(a) The semi-rigid chair form.

(b) The flexible form. This form comprises an infinite number of conformations, including various versions of boat and skew (or twisted) conformations.

In the chair conformation, steric repulsions - "non-bonded interactions" - of the substituent hydrogen atoms are at a minimum, so that this arrangement is generally thermodynamically more stable than any of the possible conformations of the flexible form. It has been calculated, and shown experimentally that the potential energy difference between forms (a) and (b) in cyclohexane and its derivatives is sufficient, in all but a few special cases, to ensure that the chair form is assumed almost exclusively[6a].

Substituted Cyclohexanes



Substituted cyclohexanes can exist in two interconvertible chair conformations. The factors influencing the relative stabilities of these two forms are of fundamental importance in the conformational analysis of cyclohexane derivatives. As can be seen in the above diagrams, a substituent X can be "axial" - a, or"equatorial" - e with respect to the molecular framework. If X is axial, it is subject to unfavourable non-bonded interactions with axial hydrogen atoms on the same side of the ring. If the substituent is equatorial, such "cross-ring" interactions are absent; i.e. the repulsive energy is smaller. Thus, in general terms, mono- and poly- substituted cyclohexanes tend to assume that chair conformation in which bulky groups are in the equatorial orientation as far as possible. There are some exceptions to this principle in a few cases involving polar substituents. Some of these are of special significance in carbohydrate chemistry, and are dealt with in the following section.

The Glycopyranose Ring [7,8,9,10]

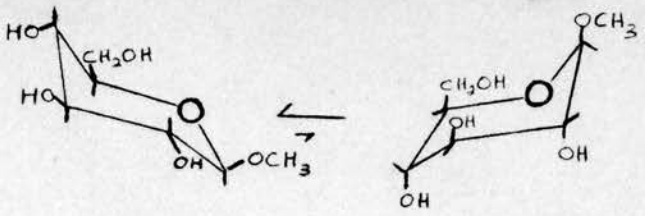
Replacement of a ring $-CH_2-$ in cyclohexanes with an oxygen atom has the following consequences:-[11,12]

(a) The C-O bonds are shorter than the C-C bonds, causing slight distortion of the ring, and bringing axial substituents on C- atoms adjacent to the O- atom into closer proximity.

(b) Two H- atoms are replaced by lone pairs of electrons.

These effects are not considerable, and the relative stabilities of the conformations of pyranose rings can be estimated using the generalisations outlined above. The validity of this treatment was supported by the classic work of Reeves[13] on the formation of cuprammonium complexes in glycoside diol systems. He assigned instability factors to the conformations of pyranose sugars based generally on the above conformational principles, and showed

experimentally that most pyranosides exist in solution in the predicted chair forms. For example, it was demonstrated that methyl β-D- glucopyranoside and its derivatives prefer the so-called C1 conformation:




C1 conformation 1C conformation (Reeves nomenclature)

Since in the C1 conformation all large substituents are equatorial, this is the predicted result.

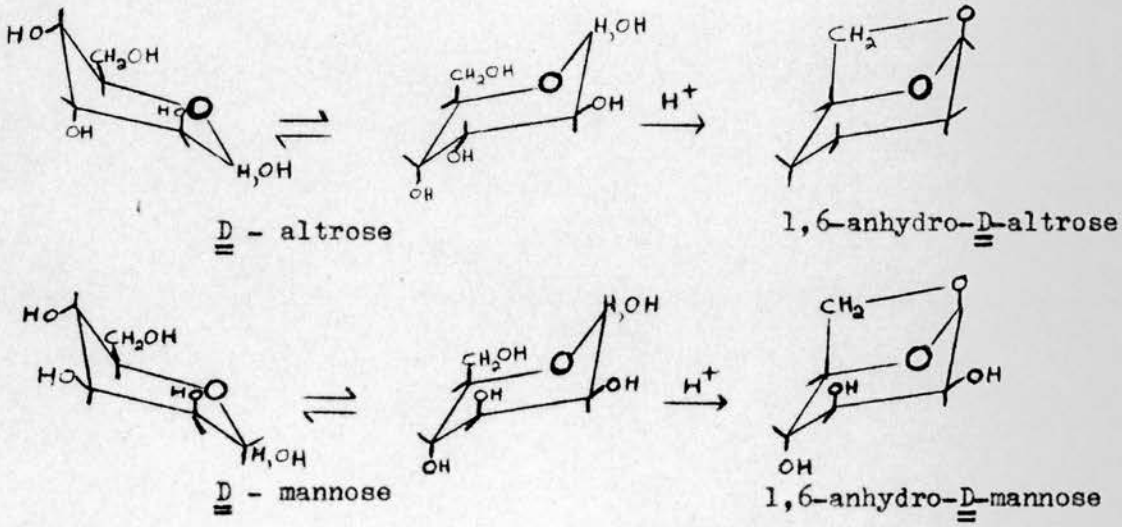
More recently, these conclusions have been largely borne out by studies on other physico-chemical properties of carbohydrates.[10] Whiffen[14] has had considerable success in correlating the optical rotations of carbohydrates with their configurational features using conformational arguments. X-ray crystallographic work has unambiguously demonstrated that in the solid state, carbohydrates exist exclusively in their predicted chair conformations.[15] Some of the most convincing evidence has undoubtedly arisen from the application of spectroscopic techniques.[16] In particular, the elegant researches of Lemieux and his co-workers[16c] in the field of N.M.R. spectroscopy are rapidly providing a firm basis for quantitative assessment of conformational instability factors. These, and studies on the equilibria of borate complexes by Angyal *et al*,[17,12] are making a major contribution towards a fuller interpretation of conformational phenomena.

To date, precise work has been done on the kinetics and equilibria of only relatively few carbohydrate reactions. However, there is a considerable body of semi-quantitative information and, in this and the following sections, some examples are given to illustrate how conformational theories have been applied

in the interpretation of the rates and equilibria of a variety of reactions.

Conformation and Equilibrium

The enzyme-catalysed equilibrium between α-D-galactose 1-phosphate and α-D-glucose 1-phosphate has been found to favour the glucose derivative[18]. This is readily explained on conformational grounds, for in the stable C1 conformation, the hydroxyl group on carbon-4 is axial (unfavoured) in the galactose phosphate and equatorial in the glucose isomer. Thus conformational analysis is a useful tool for explaining the relative stabilities of sugar isomers. A different type of example is provided by the work of Richtmeyer and his co-workers[19], who have successfully interpreted the relative extent of formation of 1,6-anhydro-hexopyranoses under acidic conditions by consideration of the conformational equilibria of their parent sugars. For example, they found that while D-altrose forms its 1,6-anhydride to an extent of about 57%, D-mannose produces less than 1% anhydro-sugar at equilibrium. They explain these results as follows:



Altrose and mannose differ only in the configuration of carbon-3. In altrose, formation of the requisite 1C conformation is favoured by the axial to equatorial shift of the 3-hydroxyl group. In mannose, on the other hand, the equatorial 3-hydroxyl group becomes axial in the 1C conformation, and meets severe non-bonded interactions with the primary alcoholic group at carbon-6. The predominance of the C1 conformation in mannose and the reluctance of this sugar to give the 1,6-anhydro compound are thus not unexpected. The prediction that the 3-deoxy analogue of these isomers (3-deoxy-D-arabino-hexose) would produce an intermediate amount of anhydro sugar was shown, by these workers, to be well founded. The D-allose:3-deoxy-D-ribo-hexose:glucose systems have been similarly examined.

In an interesting development of these arguments, Angyal, using interaction energies calculated from his borate-complex experiments, has successfully accounted for the equilibria results in a quantitative sense[12].

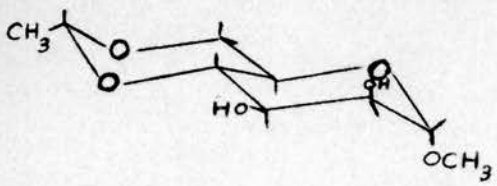
Conformation and Reactivity

The simplest measure of the reactivity of a chemical system is its rate of reaction. Chemical kinetics has long been used as a means of investigating reaction mechanism[20], and for a large number of systems, a deeper understanding of both electronic and steric factors has been achieved. In the interpretation of kinetic results, conformational analysis has proved invaluable for the elucidation of steric aspects. Carbohydrate chemistry provides a number of examples in which the relation between reaction rate and conformation is well illustrated.

Selective Esterification of Equatorial Groupings

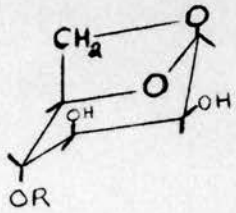
When a reaction can proceed, under a given set of conditions, by two or more pathways, product analysis gives a useful measure of the relative

reactivities among these pathways. In their work on the esterification of mannose derivatives, Aspinall and Zweifel[21] have shown that in the presence of one mole of reagent, the compounds examined esterify predominantly at a hydroxyl group which is equatorially orientated. For example:




Methyl 4,6-O-ethylidene-α-D-mannoside

I




1,6 anhydro-D-mannopyranose (R=H) and its 4-methyl ether ($R=CH_3$)

II and III

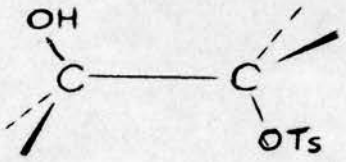
Compound I, on treatment with one mole of toluene-p-sulphonyl chloride in pyridine, gives the 3-ester in good yield, while compounds II and III both tosylate* predominantly in position 2. It is seen that the transition state for tosylation of an axial hydroxyl group meets with severe cross-ring interactions; such interactions are absent for an equatorial grouping, so that esterification is more rapid in such positions. Of further interest is the methylation of 2-O-tosyl-1,6-anhydro-D-mannose. The higher reactivity of the 4-hydroxyl toward methyl iodide may result from the greater repulsion of reagent molecules approaching the 3-hydroxyl from the "topside" of the ring; however the electronic influence of the substituent tosyl group may also contribute.

Conformational arguments thus explain many of the differences in reactivities which have been observed during preparative studies.

* form tosyl (i.e. toluene-p-sulphonyl) esters

The Rates of Formation of 3-Membered Epoxides

Methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside, on refluxing for two hours in N-NaOMe/MeOH, reacts smoothly to give the 2,3-anhydro-mannoside; it is relatively stable under mild alkaline conditions, however, and can be filtered unchanged through an untreated alumina column[22]. On the other hand, methyl 4,6-O-benzylidene-2-O-tosyl-α-D-altroside is highly sensitive to alkali[23]. It is readily converted to the 2,3-anhydro-alloside on passing through alumina; even acid-treated alumina allows the cyclisation to occur. The interpretation of these results hinges on the stereochemical requirement for epoxide formation, namely a trans-planar arrangement of the reacting hydroxyl and tosyl groupings[3a, 5a].

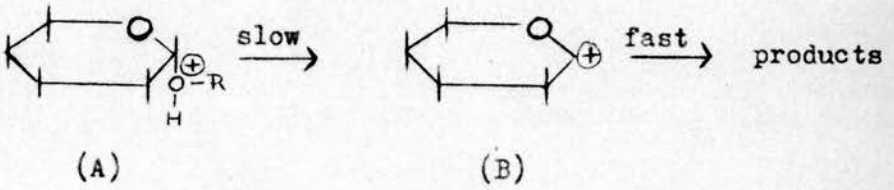



4,6-O-Benzylidene derivatives of compounds with the gluco and altro configuration exist as rigid trans-decalin-type conformations. The substituents on carbons 2 and 3 are oriented equatorially for the glucose derivatives, and axially for the altrose compounds. Thus, the reactive groups in the 2-O-tosyl-altroside have the required trans-planer arrangement in the ground state of the molecule. The high reactivity of the ester is therefore to be expected. For the glucose system, however, the pyranose ring must change to the energetically disfavoured flexible conformation prior to reaction. A similar system is discussed in detail in Part I of this work.

Examination of the literature reveals that precise kinetic studies have been directed, almost entirely, towards an understanding of reactions involving carbon-1, viz. mutarotation, glycoside hydrolysis, anomerisation, etc. While there is still some disagreement as to the detailed mechanism and stereochemistry of these reactions, conformational analysis offers interpretation of otherwise inexplicable results. Glycoside hydrolysis will be discussed as an example.

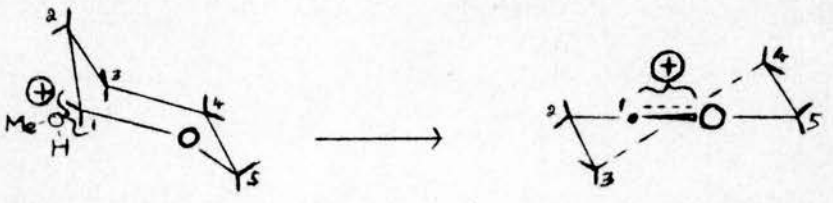
Glycoside Hydrolysis in Acidic Media

There is evidence[24] that a protonated glycopyranoside molecule (A) forms a positively-charged cyclic structure (B) during the rate-determining stage of acid-catalysed glycoside hydrolysis.




(A) (B)

Edward[25] has proposed that the intermediate cation is stabilised by conjugation with the ring oxygen. This requires that the bond between carbon-1 and the ring oxygen develops some double-bond character, and that carbons 2, 1 and 5, and the ring oxygen lie in one plane. The stereochemical implications of the conversion of the stable C1 conformation to the half chair form can be seen from the following diagrammatic scheme:



From examination of models, it is clear that cis-orientated groupings on carbons 4 and 5 (and also on carbons 2 and 3) tend to become eclipsed in the highly strained half-chair conformation. Hence achievement of the intermediate ion will be hindered by the interaction of bulky groupings on carbons 2, 3, 4 and 5. This argument is supported by the evidence[10,26,27]:

1. Pentopyranosides > 6-deoxyhexopyranosides > hexopyranosides > heptopyranosides, as regards rate of glycoside hydrolysis.

2. The greater reactivity of 2- and 3-deoxy as well as 2,3-dideoxy glycopyranosides may be due, at least in part, to this effect.

As the C1 chair form changes to the intermediate half-chair, axial groups on the same side of the ring tend to move apart. The presence of bulky axial substituents on carbons 2 and 4, and on carbons 3 and 5 might therefore be expected to favour this transformation. Examination of kinetic data reported by various workers[26,27] shows that the order of reactivities glucosides < mannosides and galactosides < gulosides for the hexopyranosides, and xylosides < arabinosides < lyxosides for the pentopyranosides agrees with the prediction that stabilities of glycosides to acid hydrolysis increase as the number of axial substituents in the C1 conformation is decreased.

Similar arguments have been applied in the interpretation of the solvolysis rates of poly-O-acetyl-glycopyranosyl halides[28], and of the anomerisation and ester-exchange rates of glycopyranose penta-acetates[10,29]. The above discussion underlines the importance of considering the stereochemistry of intermediates and transition states in the interpretation of kinetic results.

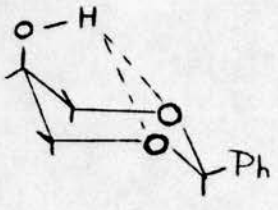
While molecules generally tend to assume a conformation in which steric compression is at a minimum, other intramolecular forces may also participate in determining conformational stability. These forces, which may arise from

dipole interactions or hydrogen bonding, often act in opposition to the steric influences, tending to stabilise conformations which are apparently disfavoured. The effects of these two factors, and the relative magnitudes of their influence on reactivity must therefore be examined.

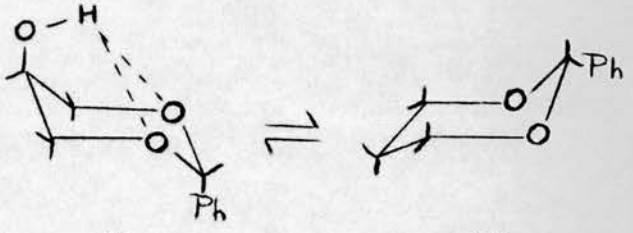
Hydrogen Bonding

The significance of hydrogen bonding in carbohydrate chemistry has been discussed at some length by Foster[8]. Hydrogen bonding may play a dual role in terms of its effect on reactivity:

(a) Conformational stability is often determined to some extent by hydrogen bonding.




IV




V VI

Spectroscopic measurements have shown that in dry carbon tetrachloride solution, cis-1,3-O-benzylideneglycerol (IV), is completely hydrogen-bonded, while the trans-isomer exists as an equilibrium mixture of the bonded form V, and the sterically favoured non-bonded form VI[8a]. It is likely that intramolecular H-bonding will be weakened in protic solvents, and examination of preferred conformations in aqueous solution by Nuclear Magnetic Resonance Spectroscopy should provide useful information on this aspect.

(b) Reactivity of a hydroxyl group towards electrophilic reagents (e.g. esterifying agents) may be enhanced by intramolecular bonding through its hydrogen atom, presumably due to increase in the basicity of the oxygen atom. The fact that in 1,4:3,6 dianhydro-D-glucitol, the "endo" 5-hydroxy group

tosylates preferentially to the sterically less hindered "exo" 2-hydroxyl can thus be explained through hydrogen bonding of the 5-grouping with a ring oxygen.

In a more recent study by Spedding[30], an attempt has been made to assess the relative strengths of internal hydrogen bonds in 4,6-O-acetal sugar derivatives. There is some difficulty, however, in interpreting the infra-red spectroscopic data.

Dipole Effects

The full import of dipole effects in carbohydrates is not yet known, though considerations of this kind must be invoked to explain a number of anomalies concerning the contribution to conformational stability of substituents on carbon-1. The α-anomers of glycosides and glycosyl esters in the C1 conformation have a bulky axial group at carbon-1. The greater stability of these compounds as compared with their β-analogues appears surprising, and the concept of dipolar interactions has been introduced into carbohydrate chemistry[16c,25] to explain this apparent anomaly. Interactions arising from the dipoles due to the ring oxygen and the anomeric oxygen must therefore be considered in all cases where conformational stability plays a part.

Electronic Effects

It is clear that knowledge of electronic behaviour is a fundamental requirement for the interpretation of relative reactivities. For complex organic molecules, such as sugars, precise evaluation of electronic effects is impossible, but a relative measure of their contribution may be estimated by correlation of experimental results from simpler, though allied systems.

The electron-withdrawing properties of acetal groups $\rangle C\langle^{OR}_{OR'}$ have been recognised for some time. In the aldoside series, there is some evidence that the hydroxyl group on carbon-2 has a relatively high dissociation constant[31]. This is commonly ascribed to the influence of the adjacent acetal linkage, and the abnormal reactivity of substituents on carbon-2, a well-known phenomenon, is often related to the "acetal effect". It is therefore important to take into account the electrophilic properties of the glycoside link when considering the relative reactivities of adjacent groupings. For example, replacement of the -OR grouping in glycosides by hydrogen, to give 1,5-anhydro-alditols, gives rise to two effects:

1. A steric effect due to substitution of a bulky -OR group ~~for~~ by the smaller H atom.

2. An electronic effect due to replacement of the acetal link with an ether-type structure with reduced electrophilic properties.

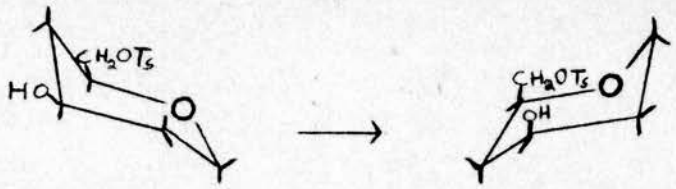
Thus, comparison of glycosides and analogous 1,5-anhydro-glycitols demands a knowledge of the relative magnitudes of these two effects.

With a view to evaluating the relative contributions of the various factors discussed above, a suitable carbohydrate reaction was sought for quantitative studies. An ideal system for these purposes was found in the well-known reaction of certain tosyl esters with alkali to yield cyclic oxides[33]. Glycopyranosides tosylated in position 6 and having a 3-hydroxyl group cis orientated with respect to carbon-6 react under alkaline conditions to give 3,6-anhydro compounds.

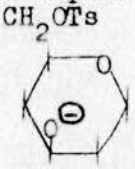
Study of this reaction offers two obvious advantages:

1. A large number of hexopyranosides possess the required stereoconfiguration for the reaction.

2. Due, in part, to the large $-CH_2OTs$ grouping on carbon-6, the great majority of these compounds exist largely in the C1 conformation. Conversion to the 1C conformation will thus be necessary to bring the reacting centres together.




**Ground-state conformation** **"Reactive" conformation**

The kinetics of cyclisation of a range of 6-tosylates has been studied in this Department by Baker[34] and Inglis[35]. By analogy with the reaction of ethylene chlorohydrin with alkali[36], it was expected that the cyclisation would proceed through an intermediate ion 

, and first order kinetics with respect to each reactant (ester and base) were anticipated. The reaction scheme could then be represented by:

$$T + OH^- \rightleftharpoons T_3^- + H_2O; \text{ and } T_3^- \xrightarrow{k} \text{products},$$

where T represent unionised ester, and $T_3^-$ the ionised species. Then the overall rate of reaction $= k[T_3^-] = kK[T][OH^-] = k_2[T][OH^-]$ where $k_2$ is a second-order rate constant. Thus second-order kinetics are anticipated if the degree of ionisation of the ester is small. It was found that the reaction was most conveniently followed at 20-25°C. in a large excess of alkali, good first-order kinetics being observed. The second-order rate constant was

then calculable by dividing the first-order value ($k_1 = k_2[OH^-]$) by the hydroxide concentration. The technique used was based on the observation that the molecular extinction coefficients of the 6-O-tosyl esters of hexosides at 265mμ lie about 0.4 $\log_{10}$ units above that of tosylate ion. Using this difference, and assuming a linear optical density/concentration relationship, a suitable method for determining first-order rate constants for the cyclisation of the 6-O-tosyl esters (≡formation of tosylate ion) in excess alkali was devised. The procedure is described in detail with respect to the analogous formation of 3-membered epoxides in Part I of this thesis.

In some preliminary investigations on a range of compounds, Baker showed that in most cases, a definite variation in the derived second-order rate constant ($k_1/[OH^-]$) with increase in hydroxide concentration was evident. This variation, which persisted at constant ionic strength (3M in sodium chloride), was interpreted in terms of reaction, to a greater or lesser extent, via a di-anion[34,35]. The reaction scheme for the analogous formation of 2,3-epoxides is examined by a similar treatment on page 51 of this thesis.

As a precaution, Baker examined the stability of the 6-O-tosyl esters in 3-molar sodium chloride solution in the absence of sodium hydroxide. Slow formation of tosylate ion occurred at various rates, determined spectrophotometrically at 265 mμ. The extreme slowness of the reactions made accurate rate measurements impossible, but a more detailed investigation of the reaction of the 6-O-tosyl esters in the absence of alkali seemed desirable, and this forms the basis of the work described in Part II.

The conformational aspects of the kinetic results are of particular interest. Inglis[35] obtained second-order rate constants of cyclisation ($k_2$) for a number of 6-tosylates by working at low hydroxide concentrations, when

the extent of reaction via di-anions is negligible. The results may be summarised as follows:

1. $\frac{k_2(\alpha\text{-gal})}{k_2(\alpha\text{-glu})} \simeq \frac{k_2(\beta\text{-gal})}{k_2(\beta\text{-glu})} \simeq 8$; 2. $\frac{k_2(\alpha\text{-glu})}{k_2(\beta\text{-glu})} \simeq \frac{k_2(\alpha\text{-gal})}{k_2(\beta\text{-gal})} \simeq 1.5$;

3. $\frac{k_2(\alpha\text{-gal})}{k_2(\alpha\text{-glu})} > \frac{k_2(2\text{-deoxy-}\alpha\text{-gal})}{k_2(2\text{-deoxy-}\alpha\text{-glu})}$;

4. $\left.\begin{matrix} k_2(\text{gluol}) \simeq k_2(2\text{-deoxy-gluol}) \\ k_2(\text{galol}) > k_2(2\text{-deoxy-galol}) \end{matrix}\right\} >> k_2(\text{glycosides})$

5. $\frac{k_2(2\text{-deoxy-}\alpha\text{-gal})}{k_2(2\text{-deoxy-}\beta\text{-gal})} > \frac{k_2(\alpha\text{-glycoside})}{k_2(\beta\text{-glycoside})} > \frac{k_2(2\text{-deoxy-}\alpha\text{-glu})}{k_2(2\text{-deoxy-}\beta\text{-glu})}$

where α-glu, α-gal ≡ methyl 6-O-tosyl-α-D-glucoside, methyl 6-O-tosyl-α-D-galactoside, etc. gluol = 6-O-tosyl-1,5-anhydro-D-glucitol; galol = 6-O-tosyl 1,5 anhydro-D-galactitol etc.

Relationships 1. and 2., considered individually, may be explained on steric grounds. In the C1 conformation, galactosides differ from glucosides in the orientation of the hydroxyl on carbon-4; thus conversion to the 1C conformation is assisted, for galactosides, by the axial to equatorial transition of this group. Similarly the C1 to 1C transformation is energetically favoured for α-anomers. The expression 3. also is expected on steric grounds, substitution of hydrogen for the 2-hydroxyl favouring the glucoside transition more than that of the galactoside. The wide difference between the rate-constant ratios 1. and 2. is at first sight surprising, since an axial substituent at $C_{(1)}$ in the 1C conformation is subject to compression from $C_{(6)}$ and $O_{(3)}$, and might therefore be expected to be less favoured sterically than an axial hydroxyl at $C_{(4)}$. It is now considered that this apparent

discrepancy is due to stabilisation of axial methoxyls at $C_{(1)}$ by dipole-dipole interactions (c.f. p. 12). Calculations by Inglis[35a] using a number of simplifying assumptions indicate that such arguments are at least plausible, and that the interaction energies are of the right order of magnitude. It is possible that some destabilisation of equatorial methoxyls at $C_{(1)}$ may also arise from dipole effects, contributing to the low value of the ratio $k_2$ α glycosides/$k_2$ β glycosides.

An alternative interpretation for such deviations from steric predictions is offered by Newth[33]. He considers that "passing interactions" (i.e. energy barriers between the stable and the reactive conformation arising from the "passing" of adjacent cis groupings during conformational change) are involved at a rate-determining stage in the reaction scheme. Theoretical considerations, together with some circumstantial evidence suggest that these interactions are unlikely to be of much significance in determining reactivity. Inglis has commented that the ratio $k_2$(α-glycoside)/$k_2$(β-glycoside) should be much lower than the analogous $k_2$(2-deoxy-α-glycoside)/$k_2$(2-deoxy-β-glycoside) ratio if passing interactions are to be considered important, and this is certainly not true for glucosides (equation V). Passing interactions are discussed in some detail in relation to the cyclisation of secondary tosylates in Part I of this thesis.

The interplay of steric and dipole effects with the electronic influence of neighbouring groups is illustrated by expression 4. Replacement of a glycosidic methoxyl group by a hydrogen atom is accompanied by elimination of the steric and dipole effects which are characteristic of glycosides. The net result is that 1,5-anhydro-glycitol derivatives show greater reactivity.

The change from acetal to ether linkage is unlikely to have much effect on the ionisation of the 3-hydroxyl group. The ionisation of this hydroxyl is, however, increased by the inductive effect of a vicinal hydroxyl. This is demonstrated clearly by comparison of the 1,5-anhydro-galactitol derivative with its 2-deoxy analogue, the latter being less reactive. The reactivities of the corresponding gluco-compounds, however, are similar, the steric effect of replacing the 2-hydroxyl by hydrogen being presumably much greater than for the galactose compounds, and acting in opposition to the electronic effect.

In view of the useful results obtained from these kinetic studies, the investigations were extended to cover the reactivities of secondary tosylates under alkaline conditions. The reaction of secondary tosylates having a trans-vicinal hydroxyl to yield 3-membered epoxides is well-known[33]. In some preliminary experiments by the writer, the reaction of methyl 2-O-tosyl-α-D-glucoside in excess alkali was examined using the Baker/Inglis technique. A rapid increase in the absorption at 265mμ showed the occurrence of a side-reaction. Some experiments carried out in an attempt to establish the nature and products of this side-reaction are described in Part III; it seems likely that the reaction involves ionisation of the hydroxyl group on $C_{(4)}$.

The 4,6-O-ethylidene derivatives of a series of secondary tosylates were therefore prepared, and their reactions in alkali examined kinetically. The preparation and kinetic investigation of these compounds are described and discussed in the following section.

# PART I

## The Rates of Formation of Some Hexoside 2,3 - Epoxides

As previously mentioned, the alkaline cyclisation of methyl 2-O-tosyl-α-D-glucoside was complicated by the observation of a side-reaction which appeared to involve ionisation of the hydroxyl on carbon-4. Smooth conversion to the 2,3-epoxide has however been reported for a large number of secondary tosylates protected by a 4,6-O-acetal grouping. Compounds of this type were therefore chosen for kinetic study, the incorporation of another 6-membered ring providing the additional advantage of restriction, due to the rigid "trans-decalin arrangement" of the number of possible conformations for the transition state. While no kinetic work has been reported for the cyclisation of such 4,6-O-acetal tosylates, it is of interest that Newth, in his review on anhydro-sugars[33], interpreted differences in the case of cyclisation of methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside and 4,6-O-benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol in terms of "passing interactions" (c.f. p. 60 ). This approach is dealt with in detail during the discussion of kinetic results.

While the benzylidene acetal has been the group most widely used for the protection of positions 4 and 6, this grouping was considered undesirable for the present work, partly because of the U.V. light absorption associated with the benzene ring, and partly because of the low solubility of this class of compound in aqueous systems. 4,6-O-Ethylidene derivatives were therefore prepared, in many cases via their 4,6-O-benzylidene analogues. Six compounds in all were prepared and examined kinetically, these being the six mono-tosylates of methyl 4,6-O-ethylidene-α- and -β-D-glucoside and 4,6-O-ethylidene-1,5-anhydro-D-glucitol.

## (A) The Preparation of Some 4,6-O-Ethylidene 2- and 3-O-Tosyl Derivatives of Compounds with the D-Glucose Configuration

### Discussion

Sulphonyl esters of sugars have found wide application in synthetic carbohydrate work and optimum conditions for the preparation of a large number of such esters have been carefully investigated by numerous workers. The syntheses and reactions of carbohydrate sulphonyl esters have been well reviewed in a comprehensive article by Tipson [37]. It is now widely accepted that sulphonyl esters are most conveniently prepared by reaction with the appropriate sulphonyl chloride in the presence of an organic base such as pyridine. By control of reaction conditions, selective esterifications are often possible, enabling many monosulphonates to be prepared directly from a simple derivative of the parent sugar.

In the syntheses reported in this section, the required mono esters were prepared either by selective esterification or de-esterification procedures, or by protection of groupings required to remain unsubstituted, sulphonylation of the remaining free hydroxyl, and subsequent removal of the protective groups. Toluene-p-sulphonyl chloride is readily available as a pure crystalline solid; it reacts with carbohydrates to form esters which are often crystalline materials with sharp melting-points. For these reasons, much of the research on sugar sulphonates has been carried out using tosyl esters. These derivatives were therefore selected for kinetic study, the preparations of many of the required compounds and precursors being already described in the literature (e.g. reference 38).

Using D-glucose as a starting point, a number of pathways to the required compounds were investigated. These are outlined in table form on the double-page insert overleaf.

# Table of Routes Used and Attempted for the Preparation of Some 4,6-O-Ethylidene 2- and 3-O-Tosyl Derivatives.

D-Glucose

2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide

1,2:5,6-di-O-isopropylidene-D-glucofuranose.

1,2:5,6 di-O-isopropylidene-3-O-tosyl-D-glucofuranose.

2,4,6-tri-O-acetyl-3-O-tosyl-α-D-glucopyranosyl bromide

Methyl 2,4,6-tri-O-acetyl-3-O-tosyl-β-D-glucoside

3-O-tosyl-1,5-anhydro-D-glucitol

4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol

Methyl 3-O-tosyl α/β-D-glucosides

Methyl 3-O-tosyl-β-D-glucoside

Methyl 2,3,4,6-tetra-O-acetyl-β-D-glucoside

Methyl-β-D-glucoside

Methyl 4,6-O-ethylidene-β-D-glucoside

Methyl 4,6-O-ethylidene-β-D-glucoside-3-nitrate

Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside-3-nitrate

Methyl 4,6-O-benzylidene-β-D-glucoside

Methyl 4,6-O-benzylidene-2-O-tosyl-β-D-glucoside

Methyl α-D-glucoside

Methyl 4,6-O-benzylidene-α-D-glucoside

Methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside

Methyl 4,6-O-benzylidene-2,3-di-O-tosyl-α-D-glucoside

Methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside

4,6-O-benzylidene-2-O-benzoyl-1,5-anhydro-D-glucitol

4,6-O-benzylidene-2-O-benzoyl-3-O-tosyl-1,5-anhydro-D-glucitol

4,6-O-benzylidene-2,3-di-O-tosyl-1,5-anhydro-D-glucitol

4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol

Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside.

Methyl 4,6-O-ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol

Methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside

Methyl 4,6-O-ethylidene-3-O-tosyl-β-D-glucoside

Methyl 4,6-O-benzylidene-3-O-tosyl-α-D-glucoside

Methyl 4,6-O-ethylidene-3-O-tosyl-α-D-glucoside

Methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside

Ennor and Honeyman[39] have prepared methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside by the reductive denitration of methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside-3-nitrate using hydrazine hydrate. In the present work, the compound was prepared from the readily-available 4,6-O-benzylidene analogue by "transethylidenation"[40], the benzylidene ring being replaced by ethylidene on reaction, under acidic conditions, with excess paraldehyde. Methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside has been prepared by a number of workers[41] by direct tosylation of methyl 4,6-O-benzylidene-α-D-glucoside. Chromatographic analysis of the products of one such tosylation revealed that mono-esterification occurred exclusively at the 2-hydroxyl[41c]. In the present work, two crystallisations from an ethyl acetate/light petroleum mixture were generally sufficient to yield the pure 2-tosylate.

Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside

This compound was prepared via the 3-nitrate, as described by Dewar and Fort[42]. Selective removal of the 2-nitrate group from methyl 4,6-O-ethylidene-β-D-glucoside-2,3-dinitrate was successfully effected using sodium nitrite in aqueous ethanol, as described for the α-anomer[40]. A sample of methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside was also prepared from the 4,6-O-benzylidene analogue by transethylidenation. The benzylidene derivative, prepared along with methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside, was best isolated by adsorption chromatography on neutral alumina. In addition to its conversion to the known ethylidene analogue, the 2-tosylate was characterised by its cyclisation, during the course of column chromatography on partly-neutralised alumina, to give methyl 4,6-O-benzylidene 2,3-anhydro-β-D-mannoside, m.p. 182-183°. The yields of tosyl esters obtained by direct tosylation were considerably reduced by the

formation of a sparingly soluble complex of methyl 4,6-O-benzylidene-β-D-glucoside with pyridine hydrochloride. This complex, apparently analogous with a similar crystalline 1:1 triphenylcarbinol/pyridine hydrochloride complex,[43] was sparingly soluble in most solvents, and crystallised well from acetone. Chemical analysis, as well as gravimetric and titrimetric analysis of its constituents, showed the complex to be 1:1, though optical rotation measurements suggested that the complex dissociated in dilute pyridine solution.

Attempts to avoid precipitation of the complex by the use of co-solvents have so far proved unsuccessful, due either to the inadequacy of the solvent (acetone, dimethyl sulphoxide), or to side reactions involving tosyl chloride (dimethyl formamide). The use of bases other than pyridine also proved fruitless. Some success was achieved using α-picoline (2-methyl pyridine) as proton acceptor, but the extreme slowness of the reaction, coupled with slow consumption of tosyl chloride (presumably to form 2-pyridyl methyl sulphone (s), c.f.44), rendered the method impracticable.

A further unexpected feature of the direct tosylation of methyl 4,6-O-benzylidene-β-D-glucoside concerns the relative amounts of the two mono-tosylates which were formed. Previous workers have described the preferential tosylations in pyridine of the 4,6-O-benzylidene derivatives of methyl α-D-glucoside[41], and 1,5 anhydro-D-glucitol[51] (position 2), and of methyl α-D-galactoside[45,46] (mainly position 3). The proportionately large amounts of 3-tosylate formed in the tosylation of the β-glucoside derivative were thus not anticipated, and an interpretation of the above results is now tentatively offered.

In tosylations involving pyridine, the esterifying agent is very likely to be the cation  [47], and tosylation rates might be expected to be enhanced or retarded by the presence, in the alcohol moiety, of adjacent

electron-donating, or electron-withdrawing groups respectively. Thus the selectivity observed in the tosylation of the methyl glycosides may be explained as follows. The electron-withdrawing glycoside link will tend to deactivate the 2-hydroxyl towards tosylation. Hydrogen-bonding of a hydroxyl, on the other hand, will tend to increase its basicity, and hence its reactivity (c.f. tosylation of 1,4:3,6-dianhydro-D-glucitol[8]). Since it has been shown that a hydroxyl group hydrogen bonds most readily to a vicinal cis-oriented substituent[30], activation of the 2-position of α-glycosides, and of the 3-position in galactosides towards tosylation is anticipated. If the hydrogen-bonding influence is considered to be stronger than that of the glycoside link, the selectivity observed in the methyl glucosides and galactosides may be explained. The preferential tosylation of the 1,5-anhydro-glucitol derivative in position-2 shows, however, that other influences, probably steric in origin, must participate.

Methyl 4,6-O-ethylidene-3-O-tosyl-α-D-glucoside

This compound, previously prepared by selective removal of the nitrate group from its 2-nitrate[39], was made, in this work, by the usual transethylidenation technique. The benzylidene analogue, also reported by Honeyman and his associates[39,40], was prepared, for the present work, by selective removal of the 2-O-tosyl group from methyl 4,6-O-benzylidene-2,3-di-O-tosyl-α-D-glucoside with sodium methoxide/methanol/chloroform[40]. A further sample was isolated by fractional crystallisation of an anomeric mixture of methyl 4,6-O-benzylidene-3-O-tosyl-α-and -β-D glucosides, prepared from 3-O-tosyl-di-O-isopropylidene-D-glucose[48]. A wide discrepancy in the optical rotations of the two samples indicated that the sample prepared from the ditosylate was probably impure.

Methyl 4,6-O-ethylidene-3-O-tosyl-β-D-glucoside

This compound was prepared by transethylidenation of methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside. Oldham and Oldham[49] have described a crystalline material, m.p. 174-176 (dec.) $[\alpha]_D$ = -93.3 (c 5.6 in $CHCl_3$), obtained on benzylidenation of the reaction products of methyl 2,4,6-tri-O-acetyl-3-O-tosyl-β-D-glucoside with methanolic sodium methoxide. This compound they claimed, not unreasonably, to be methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside. However, a low methoxyl analysis, and the failure of this compound to methylate (purification during the attempted methylation raised the m.p. to 179-182°) caused some concern. In the present work, attempts to repeat the above experiments were unsuccessful, and methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside, prepared by direct tosylation (p. 34 ) and from an anomeric mixture (p. 37 ), displayed physical properties quite different from those of the Oldham compound (viz. m.p. 158-159°, $[\alpha]_D$ -81.6). Reaction under alkaline conditions to give methyl 4,6-O-benzylidene-2,3-anhydro-β-D-alloside[48], and conversion, by debenzylidenation and acetylation, to yield methyl 2,4,6-tri-O-acetyl-3-O-tosyl-β-D-glucoside[48] provided firm chemical evidence for the structure of the compound prepared in this laboratory. The 3-tosylate methylated smoothly to produce methyl 4,6-O-benzylidene-3-O-tosyl-2-O-methyl-β-D-glucoside, m.p. 134-136°, in good agreement with the figure quoted by Oldham and Oldham, who finally obtained this compound by another route.

Suspecting incomplete deacetylation in the Oldhams' work, methyl 4,6-O-benzylidene-3-O-tosyl-2-O-acetyl-β-D-glucoside was prepared. This compound gave a good analysis, and had physical constants: m.p. 181.5-183°, $[\alpha]_D^{20}$ -101.7 (c 0.226 in $CHCl_3$). It seems clear that the Oldham compound was an impure specimen of the 2-O-acetyl derivative.

4,6-O-Ethylidene-2-O-tosyl-1,5-anhydro-D-glucitol

1,5-anhydro-D-glucitol was readily prepared via its tetra-acetate by Raney Nickel catalysed hydrogenation of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide[34]. The 4,6-O-benzylidene derivative, synthesised according to Zissis and Richtmeyer[50], tosylated selectively in the 2-position, giving 4,6-O-benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol in good yield[50,51]. Transethylidenation gave a crystalline product which, after crystallisation from chloroform/petroleum ether and methanol, analysed well for 4,6-O-ethylidene-2-O-tosyl-1,5-anhydro-D-glucitol.

4,6-O-Ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol

The preparation of the immediate precursor of the required compound, 4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol, was approached in three ways:-

a) Partial hydrogenation of 2,4,6-Tri-O-acetyl-3-O-tosyl-α-D-glucopyranosyl bromide

The reduction of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide with lithium aluminium hydride to 1,5-anhydro-D-glucitol is well known[52]. Since a number of $LiAlH_4$ reductions of sugar sulphonate derivatives have been carried out with little or no desulphonylation[53], this technique was applied to the reduction of 2,4,6-tri-O-acetyl-3-O-tosyl-α-D-glucopyranosyl bromide. This compound, prepared directly from 1,2:5,6-di-O-isopropylidene-3-O-tosyl-D-glucofuranose by the method of Freudenberg and Ivers[54], underwent rapid reaction with $LiAlH_4$ in ether/tetrahydrofuran to yield a syrup from which some 3-O-tosyl-1,5-anhydro-D-glucitol was isolated as the 4,6-O-benzylidene derivative. Since only 14mg. of 3-tosylate were obtained from 2g. of starting material, the method was rejected as impracticable.

b) Partial Detosylation of 4,6-O-Benzylidene-2,3-di-O-tosyl-1,5-anhydro-D-glucitol

Since 4,6-O-benzylidene-2,3-di-O-tosyl-1,5-anhydro-D-glucitol reacts in alkali to give a 2,3-epoxide with the allo-configuration[50], it seemed likely that, as in the case of the α-glucoside, the 3-tosylate would be formed as an intermediate. Reaction of the ditosylate with sodium methoxide/methanol/chloroform as described by Honeyman and Morgan[40] did, in fact, give 4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol, though in low yield. Studies on the $NaOMe/CHCl_3$ reagent showed that chloroform reacts quite rapidly with sodium methoxide, even at 0°. Dioxan was shown to be a suitable co-solvent for sodium methoxide reactions involving methanol-insoluble materials.

c) From 4,6-O-Benzylidene-2-O-benzoyl-1,5-anhydro-D-glucitol

The products of the benzoylation of 4,6-O-benzylidene-1,5-anhydro-D-glucitol were found to be readily resolvable by chromatography on activated silica gel. Both mono-benzoates were formed, there being significantly more of the 3-benzoate (contrast Newth[51]). Tosylation of each produced the two known tosylate benzoates. After consideration of reagents active in the fission of carboxylic esters at pH's approaching neutrality[55], hydrazine was selected as a suitable and convenient reagent for debenzoylation in the presence of sulphonyl esters (c.f. ref. 39). It was found, in fact, that 4,6-O-benzylidene-3-O-tosyl-2-O-benzoyl-1,5-anhydro-D-glucitol, on refluxing in ethanolic hydrazine hydrate, gave the required 4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol without perceptible detosylation. Conversion to the crystalline 4,6-O-ethylidene analogue by transethylidenation completed the synthesis.

## Chromatography

Since most of the compounds prepared during synthetic work were relatively hydrophobic, a large proportion of the paper chromatographic techniques normally used in carbohydrate chemistry were found inapplicable. Two methods for resolving highly substituted sugar derivatives were studied, and found useful and complementary - paper chromatography on dimethyl sulphoxide-impregnated paper[56], and thin-layer chromatography on alumina and silica gel[57]. The former technique, in which the mobile phase was usually diisopropyl ether, was found particularly useful for the separation of anomers, although runs of as long as 4 days were sometimes required to give complete resolution. Thin-layer chromatography, on the other hand, provided a rapid and versatile tool for following the course of column chromatography on silica and alumina, as well as for identification, and as a criterion of purity for a large number of sugar derivatives. In several cases, a semi-quantitative estimate of the relative proportions of reaction products was possible. The solvents employed in this latter technique depended, of course, upon the class of compounds being separated. As predicted from column chromatography, the solvent systems which give best resolution depend on the degree of substitution of the carbohydrate hydroxyl groups, more hydroxylated molecules in general requiring more polar solvents for elution.

## EXPERIMENTAL

Solvents and reagents used during preparative work were generally of "Analar" quality. When required, dry benzene and ether were stored over sodium, pyridine was distilled off barium oxide, and tetrahydrofuran was pretreated with lithium aluminium hydride. Solvents were removed under reduced pressure in a rotary evaporator, the water-bath temperature being controlled, usually to 40° or below.

Infra-red spectra were measured on an Infracord 137 Recording Spectrophotometer, Nujol being used for the dispersion of solid material.

### Analytical Chromatography

#### a) Paper Chromatography [56]

Good separations were achieved using dry dimethyl sulphoxide as a stationary phase, di-isopropyl ether or moist diethyl ether being employed as mobile phase. The stationary phase was introduced by passing Whatman No.1 filter paper rapidly through a 25% v/v solution of dimethyl sulphoxide in toluene, blotting off the excess liquid with filter-paper, and drying off the toluene in an oven at ca 70° for 1 minute. This procedure, repeated twice, introduced enough dimethyl sulphoxide on to the paper for chromatographic purposes. The treated paper was held between glass plates during spotting to prevent absorption of atmospheric moisture, and quickly transferred to the tank, the inside of which was kept moisture-free with activated silica gel. The chromatogram was then run, using the descending technique, for a time ranging from 1½ hours to 4 days, depending on the degree of separation required. Tosylates were readily detected by spraying the dried paper with a 1% w/v ethanolic solution of diphenylamine, and exposing to U.V. light for 5 - 10 minutes. Spots with a bright bluish fluorescence visible under the U.V. lamp were given by all the tosylates examined[58a].

b) Thin-layer Chromatography[57]

Glass plates, 20cm. by 5cm., were coated with uniform layers of silica gel or alumina (ca. 275μ thick) using the apparatus manufactured by Desaga. The adsorbant layers were activated as required by oven heating (30 minutes at 105° served for most purposes), and the plates stored in a desiccator till required. Runs were carried out by ascending chromatography, solvents and solvent mixtures being chosen empirically. For most of the separations required in this work, alumina in conjunction with benzene:ether (1:1 v/v), or silica gel with ether:benzene (3:1 v/v) were used with success, the two systems being in many cases complementary. A convenient universal spray, which appeared to show up all carbohydrate material, was found in an ethanolic solution of anisaldehyde and concentrated sulphuric acid (5% each v/v).[59] The spray, best used on silica layers, gave purple spots with sugar derivatives on heating the treated plates for 10 minutes at 120°. Diphenylamine reagent used as described for paper chromatography was conveniently employed in resolutions of sugar tosylates and nitrates.

Methyl β-D-glucoside

Rudowski[60] has claimed excellent yields of methyl β-D-glucoside by the glycosidation of glucose in methanol with alkyl orthoformates.

The following preparation, derived from his instructions, is modified according to Raymond and Schroeder[61].

A mixture of glucose (90g.), ammonium chloride (5g.) and methyl orthoformate (57.5g.) in anhydrous methanol (1000 ml.) was refluxed for 7 hours, then stood overnight at room temperature. Under these conditions, the optical rotation of the solution had reached a minimum positive value. The solution, now light brown in colour, was then concentrated in vacuo to give a thick brown syrup. After extraction with acetone and ether to remove much of the orthoformate impurities

portions of the syrup were seeded with methyl β-D-glucoside, and treated by the usual methods in an attempt to induce crystallisation. All attempts being unsuccessful, the syrup was dissolved in ethanol (250 ml), and the hot solution treated with a solution of potassium acetate (50g.) in hot ethanol (150 ml).

After removal of some potassium acetate which crystallised out on standing at room temperature, the solution was stood in the refrigerator for 2 days. The pasty white suspension of the methyl β-D-glucoside-potassium acetate complex was filtered off at the pump, washed with several portions of ice-cold ethanol, and dissolved in hot methanol (200 ml.). A hot solution of tartaric acid (16.5g.) in ethanol (150 ml.) was then poured in, and the cooled mixture filtered through a sintered glass funnel. The filtrate, after concentrating to a thin syrup and standing overnight in the refrigerator, yielded crystals of crude methyl β-D-glucoside (16.9g.). Recrystallisation from ethanol yielded pure methyl β-D-glucoside (13.8g, m.p. 105-108°) as large white prisms.

The yield under these conditions, was only slightly higher than that obtained from a methanolic-HCl anomerisation.

Methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside

Methyl 4,6-O-benzylidene-α-D-glucoside (20g.), prepared by the method of Edington[62], was dissolved in pure dry pyridine (50 ml.), and cooled in a freezing mixture to about -10°. A solution of tosyl chloride (13.5g., 1.1 mole) in dry pyridine (40 ml.) was then added dropwise with vigorous mechanical stirring, the temperature being kept at 0°, and moisture being excluded. After addition was complete, the reaction mixture was allowed to warm to room temperature, then stood at this temperature until the optical rotation of the mixture was constant (5 hours), and finally left overnight. Excess tosyl chloride was decomposed by adding water (5 ml.) dropwise, with cooling and shaking, over about 20 minutes. The pyridine

solution was now taken up in chloroform (200 ml.), and the resulting reddish solution was washed free from pyridine with excess normal sulphuric acid (ca. 2 litres). The chloroform was then washed with saturated sodium bicarbonate, and then water-washed to eliminate last traces of inorganic impurities.

After drying the solution with anhydrous sodium sulphate, the chloroform was removed under reduced pressure, and the resulting syrup rendered crystalline by rubbing with petroleum ether. The crude material (22g.) formed white powdery crystals m.p. 148-151° on one recrystallisation from ethyl acetate/petroleum ether solution. A further recrystallisation and charcoal treatment gave pure methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside (19.2g. m.p. 152.5-153°), lit: m.p.154-156°.

Treatment with N-sodium methoxide/methanol according to the procedure of Bollinger and Prins[41c] gave 82% of methyl 4,6-O-benzylidene-2,3-anhydro-α-D-mannoside, m.p. 144-146°.

Methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside

A suspension of methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside (5.0g.) in paraldehyde (25 ml.) was shaken with concentrated sulphuric acid (0.5 ml.) for 20 minutes, and the clear pale-yellow solution poured, with stirring, into petroleum ether (200 ml.) and saturated aqueous sodium bicarbonate (100 ml.). The oil produced solidified on rubbing with a glass rod to yield crude material (3.7g.). Evaporation of the petrol layer to dryness yielded a further crop (0.3g.) of crude product. Successive recrystallisations from ethyl acetate/petrol ether, ethanol, and aqueous ethanol gave methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside (3.2g.) m.p. 150-152°, $[\mu]_D^{20}$ + 83.0 (c1.02 in $CHCl_3$) lit: m.p. 150°, $[\alpha]_D^{22}$ + 82.1[39] (Found: C, 51.7; H, 5.8; S, 8.4. Calculated for $C_{16}H_{22}O_8S$: C, 51.3; H, 5.9; S, 8.6%).

Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside

a) Dewar and Fort Preparation[42]

Methyl 4,6-O-ethylidene-β-D-glucoside-2,3-dinitrate

Methyl 4,6-O-ethylidene-β-D-glucoside (5.9g.), prepared according to Dewar and Fort[42], was suspended in acetic anhydride (16 ml.) and the suspension cooled to 0°, was treated with a solution of fuming nitric acid (5.1 ml.) in acetic anhydride (16 ml.). The reaction mixture was kept at 0° with gentle stirring for one hour, and the clear solution poured into ice-water with vigorous agitation. The oily product solidified almost at once, and was removed by filtration, dried, and crystallised from methanol, and petrol ether to give methyl 4,6-O-ethylidene-β-D-glucoside-2,3-dinitrate (5.6g.) m.p. 88-89° (lit: m.p. 88-89°)[42].

Methyl 4,6-O-ethylidene-β-D-glucoside-3-nitrate

Methyl 4,6-O-ethylidene-β-D-glucoside 2,3-dinitrate (5.4g.) and sodium nitrite (3.14g.) were dissolved in a mixture of ethanol (25 ml.) and water (6.3 ml.), and the solution refluxed for 48 hours. The solvent mixture was then removed, and the residue extracted with a water-chloroform mixture. The chloroform layer was separated, dried over anhydrous sodium sulphate, and the chloroform removed to give a white solid. Crystallisation from ether gave methyl 4,6-O-ethylidene-β-D-glucoside-3-nitrate (2.9g.) m.p. 143-144° (lit: m.p. 146-147°)[42]. On recrystallisation from ethyl acetate/petrol ether, the melting-point rose to 146-147°.

Methyl4,6-O-ethylidene-2-O-tosyl-β-D-glucoside-3-nitrate

A solution of methyl 4,6-O-ethylidene-β-D-glucoside-3-nitrate (2.8g.) in dry pyridine (9.6 ml.) was shaken with tosyl chloride (3.0g.), and the mixture allowed to react at room temperature for 6 days. After the destruction of excess reagent

with water, the pyridine solution was taken up in chloroform (40 ml.), and the pyridine removed by washing with a 2-fold excess of 2N-sulphuric acid (120 ml.). The chloroform solution was then washed with sodium bicarbonate solution, and distilled water, dried over anhydrous sodium sulphate, and evaporated to dryness, yielding a syrup which solidified on rubbing with methanol. Crystallisation twice from methanol gave methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside-3-nitrate (3.2g.) m.p. 143-144° (lit: m.p. 143-144°)[42].

Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside

Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside-3-nitrate (100 mg.) dissolved in glacial acetic acid (2.0 ml.), was warmed to 50°, and shaken with portions of iron filings/zinc dust (3/1 by weight) until diphenylamine reagent [58b] showed nitrate ester to be absent (12 minutes). The mixture was filtered, the residue washed with portions of chloroform (total 20 ml.), and the combined filtrate and washings washed with distilled water to remove acid. The water washings were back-extracted with chloroform, and the total chloroform extracts were combined, dried over anhydrous sodium sulphate, and the chloroform removed under reduced pressure. The product, recrystallised twice from chloroform/petrol ether as shiny plates, was methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside (78 mg.) m.p. 152-153° (lit: m.p. 152-153°)[42].

b) Preparation from 4,6-O-benzylidene Analogue

"Transethylidenation" of the benzylidene analogue (350 mg.) in paraldehyde (3 ml.) and concentrated sulphuric acid (0.04 ml.) gave, on two crystallisations of the crude product from chloroform/petrol ether, and one charcoal treatment, methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside (190 mg.) m.p. 151-152°, identical to that obtained *via* the 3-nitrate.

Direct Tosylation of Methyl 4,6-O-benzylidene-β-D-glucoside

Methyl 4,6-O-benzylidene-β-D-glucoside (35g.), prepared by the Edington procedure[62], was dissolved in dry pyridine (100 ml.), and treated with tosyl chloride (25g.) in dry pyridine (80 ml.) as described above for the α-anomer. A white crystalline precipitate (15.0g.) formed during the reaction was removed by filtration, washed with cold dry pyridine, and subsequently examined chemically. The solution of reaction products was worked up as usual, the final chloroform solution yielding a sticky solid (16.4g.) smelling faintly of benzaldehyde. Examination of the crude reaction mixture by thin-layer chromatography on alumina with benzene:ether (1:1 v/v) indicated that the tosylates were present in the ratio of about 2:2:1 (ditosylate:3-tosylate:2-tosylate). Crystallisation from methanol (300 ml.) gave methyl 4,6-O-benzylidene-2,3-di-O-tosyl-β-D-glucoside (7.2g.) m.p. 178-180°, mixed m.p. with an authentic sample 179-181.5°.

The syrup obtained on removal of the methanol was then extracted with 1:1 benzene:petrol ether (500 ml.), and the extract and residue examined.

Residue: Crystallisation, twice from chloroform/petrol ether, and once from ethyl acetate/petrol ether gave methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside (3.2g.) m.p. 158-159°, $[\alpha]_D^{20}$ - 82.6 (c 1.126 in $CHCl_3$) (Found: C,57.4; H, 5.4; S, 6.9. $C_{21}H_{24}O_8S$ requires C, 57.8; H, 5.5; S, 7.4%). On treatment with NaOMe/MeOH, the 3-tosylate was smoothly converted to methyl 4,6-O-benzylidene-2,3-anhydro-β-D-alloside, m.p. 138°[48]. Acid debenzylidenation followed by acetylation of the syrupy intermediate gave methyl 2,4,6-tri-O-acetyl-3-O-tosyl-β-D-glucoside[48], m.p. 137-138°, $[\alpha]_D$ - 19.8 (c 1.011 in $CHCl_3$) on two crystallisations from aqueous methanol.

Extract: Removal of solvent gave a brown syrup (3.5g.) which yielded a crop of white needles (0.3gm.) on crystallisation from ethanol/benzene/petrol ether. A further recrystallisation from chloroform/petrol ether yielded methyl 4,6-O-benzylidene-2-O-tosyl-β-D-glucoside (0.25g.) m.p. 121-122°. (Found: C, 57.6; H, 5.7; S, 6.7. $C_{21}H_{24}O_8S$ requires C, 57.8; H, 5.5; S, 7.4%). The structure of this tosylate was confirmed by conversion to the known 4,6-O-ethylidene analogue (see p. 33 ). Freedom from the 3-tosylate and other impurities was demonstrated by thin-layer chromatography on alumina.

After isolation of more crystalline ditosylate (0.6g.) from the combined mother liquors, the residue was dissolved in benzene, and chromatographed on neutral alumina. On elution with mixtures of ether and benzene 1:19, 1:9, and 1:1 by volume, partial resolution of the products into ditosylate and the two monotosylates was obtained, the ditosylate emerging first, closely followed by the 2-tosylate, and the 3-tosylate in that order. Thin-layer chromatography on alumina of selected fractions showed that during elution, some overlap of the 2-tosylate band with those of the ditosylate at one end, and of the 3-tosylate at the other had occurred. Intermediate fractions, containing only traces of the di- and 3-tosylates, were therefore combined and recrystallised from chloroform/petrol ether to give methyl 4,6-O-benzylidene-2-O-tosyl-β-D-glucoside (0.74g.), m.p. 121-122°.

Further elution with 1:1 benzene:ether yielded more crystalline material which, on recrystallisation from ethyl acetate/petrol ether, gave methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside (1.22g.), m.p.158-159°.

The Crystalline Precipitate

Recrystallisation from acetone gave white needles, m.p. 158-160°, sparingly soluble at room temperature in most common solvents. The production of methyl

4,6-O-benzylidene-β-D-glucoside, m.p. 199-200°, on washing with hot water, and examination of the infra-red spectrum of the original material suggested that a stable complex between the starting-material and pyridine hydrochloride had been formed. This viewpoint was supported by the production of a crystalline precipitate with properties similar to the above on treating a solution of methyl 4,6-O-benzylidene-β-D-glucoside (0.4g.) in dry pyridine (1.0 ml.) with a saturated solution of pyridine hydrochloride in pyridine (1.0 ml.). Analysis by alkali titration, and gravimetric determination of the weight of starting material produced gave evidence of a 1:1 complex. Chemical analysis supports the above conclusions: C, 57.4; H, 6.1; N, 3.6; Cl, 8.6% $C_{19}H_{24}O_6NCl$ required C, 57.4; H, 6.0; N, 3.5; Cl 8.9%.

The optical rotation of a dilute solution (c = 1.023) of the complex in pyridine was measured, and compared with that of a solution containing an equivalent amount of methyl 4,6-O-benzylidene-β-D-glucoside (that is, assuming a 1:1 complex). The rotations (0.70 for the complex, 0.69 for the glucoside) were the same within experimental error, indicating that the glucoside/pyridine hydrochloride complex is completely dissociated in dilute pyridine solution.

Preparation of Methyl 4,6-O-benzylidene-3-O-tosyl-α-and-β-D-glucoside

Methanolysis of 1,2:5,6 di-O-isopropylidene-3-O-tosyl-D-glucofuranose

1,2:5,6 di-O-isopropylidene-3-O-tosyl-D-glucofuranose (35.5g.), prepared by the method of Freudenberg and Ivers[54], was refluxed for 10 hours in 2% dry methanolic HCl (370 ml.) under anhydrous conditions, the course of the reaction being followed by observation of changes in the optical rotation of the solution. The rotation approaching constancy, the reaction was then stopped by cooling, and excess acid neutralised with solid lead carbonate. Inorganic material was now

filtered off under suction, and excess methanol removed from the filtrate *in vacuo* to yield a light brown syrup (30.7g.). Partition chromatography on paper using 4:1:5 butanol:ethanol:water showed this syrup to be free from detosylation products.

Benzylidenation of the Anomeric Mixture

The syrupy anomeric mixture (30.7g.) was dissolved in redistilled benzaldehyde (300 ml.) and shaken for 18 hours with powdered anhydrous zinc chloride (45g.). On pouring into a mixture of saturated aqueous sodium bicarbonate (720 ml.) and petroleum ether (600 ml.), a brown oil separated, which was then removed, and washed with several portions of petrol ether to remove the bulk of the benzaldehyde. The viscous syrup was now dissolved in chloroform (250 ml.), and the solution washed twice with an ice-cold saturated aqueous solution of sodium bisulphite. The chloroform layer was then washed with sodium bicarbonate solution, dried over anhydrous sodium sulphate, and evaporated to give a mass of sticky crystals (21.4g.). A further yield of the benzylidene mixture (9.2g.) was extracted with chloroform from the zinc carbonate precipitate. Paper chromatography on filter paper impregnated with dimethyl sulphoxide indicated that the α- and β- 3-*O*-tosyl anomers were present in approximately equal amounts in the crude reaction mixture.

The fractional crystallisation of the anomers was followed by examination of the infra-red spectra of Nujol mulls prepared from samples of each fraction, the α and β anomers possessing characteristic peaks at 894 and 875 $cm.^{-1}$ respectively.

A portion of the crude product (10.0g.) was crystallised from benzene/petrol ether, chloroform/petrol ether, methanol/water and finally ethyl acetate/petrol ether to yield methyl 4,6-*O*-benzylidene-3-*O*-tosyl-β-*D*-glucoside (1.9g.) m.p.157-159°, mixed m.p. with the sample prepared by direct tosylation, 158-159°, $[\alpha]_D^{21}$ -80.6 (*c* 1.079 in $CHCl_3$).

The mother liquor of the chloroform/petrol ether recrystallisation was concentrated to dryness, two crystallisations from benzene/petrol ether yielding methyl 4,6-O-benzylidene-3-O-tosyl-α-D-glucoside (3.2g) as hard prisms m.p.160°, $[\alpha]_D^{18}$ + 31.6 (c 1.884 in $CHCl_3$) (lit: m.p. 159-160°, $[\alpha]_D$ + 32.5, c 0.9 in $CHCl_3$)[40].

The remaining crude product, on crystallisation from chloroform/petrol ether, gave more of the 3-tosylate-β-anomer (8.0g.). The residual syrup could not be induced to crystallise.

Partial Detosylation of methyl 4,6-O-benzylidene-2,3-di-O-tosyl-α-D-glucoside

Methyl 4,6-O-benzylidene-2,3-di-O-tosyl-α-D-glucoside (7.0g.) was dissolved in chloroform (90 ml) and treated, at 0°, with 2.6N-sodium methoxide in methanol (22.0 ml.) as described by Honeyman and Morgan. After working up, the crystalline product was crystallised from benzene, benzene/petrol ether and ethyl acetate/petrol ether to give methyl 4,6-O-benzylidene-3-O-tosyl-α-D-glucoside (0.72g) as soft needles m.p. 156-159°, mixed m.p. with a sample prepared as described earlier 160°, $[\alpha]_D^{18}$ + 46.6 (c 1.887 in $CHCl_3$) (lit: m.p. 159-160°, $[\alpha]_D$ = 32.5, c 0.9 in $CHCl_3$)[40]. The hard prisms and soft needles were interconvertible on crystallisation and differences in their infra-red spectra confirmed the dimorphous nature of this compound.

Methyl 4,6-O-ethylidene-3-O-tosyl-α-D-glucoside

Methyl 4,6-O-benzylidene-3-O-tosyl-α-D-glucoside (570 mg.) was converted into the 4,6-O-ethylidene analogue by the standard "transethylidenation" procedure. Three crystallisations of the crude material from chloroform/petrol ether yielded methyl 4,6-O-ethylidene-3-O-tosyl-α-D-glucoside (270 mg.) as white needles m.p. 144-146°, $[\alpha]_D^{19}$ + 63.7 (c 1.492 in $CHCl_3$) (lit: m.p.145-145.5°, $[\alpha]_D^{22}$ + 68.4, c 0.7 in $CHCl_3$)[39].

Methyl 4,6-O-benzylidene-3-O-tosyl-2-O-acetyl-β-D-glucoside

Methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside (0.3g.) was shaken with acetic anhydride (0.2 ml.) in dry pyridine (2.0 ml.) until solution had taken place, when the mixture was stood at room temperature for 20 hours. Excess reagents were removed *in vacuo* at 50°C, and the crystalline product crystallised three times from chloroform/petrol ether to yield methyl 4,6-O-benzylidene-3-O-tosyl-2-O-acetyl-β-D-glucoside (0.24 g.) m.p. 181.5-183° $[\alpha]_D^{20}$ - 101.7 (c 0.226 in $CHCl_3$) (Found: C, 57.7; H, 5.4; S, 6.9. $C_{23}H_{20}O_9S$ requires C, 57.7; H, 5.4; S, 6.7%).

Methyl 4,6-O-benzylidene-3-O-tosyl-2-O-methyl-β-D-glucoside

A solution of methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside (0.4g.) in methyl iodide (10 ml.) was heated to reflux, with the exclusion of moisture, and dry silver oxide (1.6g.) was added in portions over 90 minutes. The reaction mixture was now refluxed for a further 4 hours, the insoluble material filtered off and washed with a little methyl iodide, and the combined filtrate and washings evaporated to dryness under reduced pressure. The solid product, on crystallisation from ether/petrol ether, methanol and chloroform/petrol ether, gave methyl 4,6-O-benzylidene-3-O-tosyl-2-O-methyl-β-D-glucoside (0.20g), m.p. 134-136° (lit:135-136°)[49] (Found: C, 58.8; H, 5.8; S, 6.0. Calculated for $C_{22}H_{26}O_8S$: C, 58.7; H, 5.8; S, 7.1).

Methyl 4,6-O-ethylidene-3-O-tosyl-β-D-glucoside

Methyl 4,6-O-benzylidene-3-O-tosyl-β-D-glucoside (2.3g.) was shaken with paraldehyde (12 ml.) containing concentrated sulphuric acid (0.2 ml.) as described for the α-2-tosylate analogue. The crude product, dried over $P_2O_5$, was crystallised from chloroform/petrol ether, ethanol, ethyl acetate, and twice from benzene, to give methyl 4,6-O-ethylidene-3-O-tosyl-β-D-glucoside (1.6g.), m.p. 164-165.5° (Found: C, 51.4; H, 6.1; S, 8.0. $C_{16}H_{22}O_8S$ requires C, 51.3; H, 5.9; S, 8.6%).

4,6-O-Benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol

1,5-anhydro-D-glucitol, prepared from 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-bromide by Raney Nickel hydrogenation and deacetylation as described by Baker[34], was converted into its 4,6-O-benzylidene derivative by the method of Zissis and Richtmeyer[50]. 4,6-O-Benzylidene-1,5-anhydro-D-glucitol (10g.) was dissolved in dry pyridine (30 ml.) and treated with tosyl chloride (7.4g.) in dry pyridine (25 ml.) as described for the selective tosylation of the methyl α- and β-D-glucoside analogues. A crystalline solid, precipitated from the reaction mixture on standing overnight, was isolated by filtration, washed with pyridine, and crystallised twice from chloroform/petrol ether to yield 4,6-O-benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol (1.3g.) m.p. 177.5-178.5° (lit: m.p. 175-179°; 174-175°[50,51]). The pyridine solution, on working up, gave a further yield of the 2-O-tosyl ester (4.8g.), m.p. 178-178.5° $[\alpha]_D^{19}$ -22.7 (c 1.81 in $CHCl_3$), on two crystallisations from chloroform/petrol ether.

4,6-O-Ethylidene-2-O-tosyl-1,5-anhydro-D-glucitol

"Transethylidenation" of the above 4,6-O-benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol (2.7g.) in paraldehyde (13.5g.) and concentrated sulphuric acid (0.25 ml.) was undertaken, part of the product being precipitated from the reaction solution. Crystallisation of the crude product, twice from chloroform/petrol ether, and once from methanol, gave 4,6-O-ethylidene-2-O-tosyl-1,5-anhydro-D-glucitol (1.5g.) m.p. 157°. (Found: C, 52.5; H, 5.6; S, 9.6. $C_{15}H_{20}O_7S$ requires: C, 52.3; H, 5.8; S, 9.3%).

4,6-O-Benzylidene-2-and-3-O-benzoyl-1,5-anhydro-D-glucitol

Benzoyl chloride (3.23 ml.) in dry pyridine (20 ml.) was added dropwise to a well stirred solution of 4,6-O-benzylidene-1,5-anhydro-D-glucitol (5.8g.) in

pyridine (25 ml.) which was pre-cooled to -10° in a freezing mixture. After allowing the mixture to react overnight, excess benzoyl chloride was destroyed with water, and the residual mixture was worked up according to the procedure described for tosylations. The reaction products solidified on the removal of chloroform, and crystallisation from ethanol gave 4,6-O-benzylidene-2,3-di-O-benzoyl-1,5-anhydro-D-glucitol (0.62g.) m.p. 162-164° (lit: m.p. 162-163°)[51]. Three further crops, total weight 3.54g., were shown, by thin-layer chromatography on silica, to consist largely of the two monobenzoates. The mixture, dissolved in benzene (75 ml.), was chromatographed on activated silica gel. Elution with benzene gave a solid which, on crystallisation from methanol, gave dibenzoate (0.25g.). Benzene:ether (1:1) eluted a further band of material which, on crystallisation from methanol, gave 4,6-O-benzylidene-2-O-benzoyl-1,5-anhydro-D-glucitol (1.12g.) m.p. 133- 134° $[\alpha]_D^{22}$ + 30.4 (c 1.15 in $CHCl_3$) (lit: m.p. 133-134°)[48]. Further elution with the same solvent mixture gave 4,6-O-benzylidene-3-O-benzoyl-1,5-anhydro-D-glucitol (1.96g.) m.p. 152-153°

The structure of the 3-benzoate was confirmed by tosylation to give the known 4,6-O-benzylidene-2-O-tosyl-3-O-benzoyl-1,5-anhydro-D-glucitol, m.p. 199-200°.[51]

4,6-O-Benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol

1) From 3-O-tosyl-2,4,6-tri-O-acetyl-α-D-glucopyranosyl bromide

2.31 M-lithium aluminium hydride solution (10 ml.) in dry ether was cooled in an ice bath with vigorous mechanical stirring, and the 3-O-tosyl-bromacetate (2.0g.) in anhydrous tetrahydrofuran (40 ml.) was added dropwise at a rate which produced a slow refluxing of the ether/tetrahydrofuran solution. During the addition, moisture was excluded, and the reaction was carried out over oxygen/water-free nitrogen, as recommended by Gaylord.[53a] After the addition was complete (40 mins.),

the solution was allowed to react overnight at room temperature, and excess reagent was destroyed using ethyl acetate. N-sulphuric acid (10 ml.) was added slowly to hydrolyse the reaction complexes, followed by distilled water (40 ml.). The homogeneous solution was neutralised with solid barium hydroxide followed by barium carbonate, and the inorganic magma filtered off with the aid of celite.

The clear solution was evaporated to dryness under reduced pressure, last traces of water being removed by co-distillation with dry benzene. The sticky solid residue was then extracted continuously for five hours with 150 mls. of chloroform, a Soxhlet extractor being used. Removal of the chloroform gave a brown syrup which was shown, by means of thin-layer chromatography on silica gel with ethyl acetate, to contain at least three tosylate components. Extraction of the syrup with water (3 x 20 ml.) followed by evaporation of the combined extracts gave a syrup (230 mg.) which appear to contain only one tosylate component, but which could not be induced to crystallise.

Benzylidenation of the syrup by the usual method (benzaldehyde/zinc chloride) gave a syrup which, on rubbing with chloroform/petrol ether, partially crystallised to give an amorphous-looking solid (20 mg.) m.p. 145- 149°. Recrystallisation from ethyl acetate/petrol ether gave impure 4,6-O-benzylidene-3-O-tosyl-1,5-anydro-D-glucitol (14 mg.), m.p. 155-158°, whose chromatographic behaviour on alumina plates with 1:1 benzene ether, and infra-red spectrum compared favourably with those of samples whose preparation is described elsewhere.

2) From the 2-benzoate

4,6-O-benzylidene-2-O-benzoyl-1,5-anhydro-D-glucitol (0.84g.) dissolved in 3.5 mls. of dry pyridine was reacted overnight at 40° with tosyl chloride (0.70g.). After working up in the usual way, crystallisation of the pyridine-free reaction products from ethyl acetate/petrol ether gave 4,6-O-benzylidene-2-O-benzoyl-3-O-

tosyl-1,5-anhydro-D-glucitol (0.62g.), m.p. 191-192° ( softened 184°). (lit: m.p. 192° )[48].

Selective removal of the benzoate group was achieved by refluxing the compound (0.52g.) in ethanol (6 ml.) containing hydrazine hydrate (0.26g.) for 6 hours. The ethanol was removed at 25° under reduced pressure, the residue dissolved in chloroform (30 mls.), and the solution washed with dilute sulphuric acid, sodium bicarbonate solution, and distilled water. Removal of chloroform from the dried solution gave a white solid which, on two crystallisations from chloroform/petrol ether, yielded 4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol (0.21g.) m.p. 161°.

3) From the ditosylate

4,6-O-Benzylidene-2,3-di-O-tosyl-1,5-anhydro-D-glucitol (2.0g.), prepared by the method of Zissis and Richtmeyer[50], was allowed to react in chloroform (25 ml.) with methanolic sodium methoxide (6 ml. 2.7M) as described for the α-glucoside analogue. The crude solid reaction products were extracted with portions of hot methanol (total 50 mls.), the combined extracts cooled, and the precipitate of unchanged ditosylate removed by filtration. The solid residue, on removal of methanol, was extracted with cold benzene, and the extracted material crystallised from the same solvent to give a product (50 mg.) m.p. 155-157° as an amorphous-looking solid. Attempts to raise the melting-point by recrystallisation were unsuccessful, but the infra-red spectrum, behaviour on thin-layer chromatograms, and subsequently a mixed melting-point showed the product to be impure 4,6-O-benzylidene-3-O-tosyl-1,5-anhydro-D-glucitol. The yield was too low for preparative use.

The Reaction of Chloroform with Methanolic Sodium Methoxide

Five solutions of sodium methoxide in methanol (3.0 ml., 2.7N) were treated at 0° with 12.0 ml. portions of chloride-free chloroform. The mixtures were stood at this temperature for 27, 66, 92, 145 and 217 hours, the reaction after these times being stopped by the addition of water, and the solutions being titrated for free base and chloride ion using standard normal sulphuric acid and silver nitrate solutions respectively. The amounts of sodium methoxide consumed in these times, expressed as percentage of the initial amount were:- 31.0%, 49.5%, 56.0%, 67.3% and 75.8% respectively.

Use of dioxan/sodium methoxide/methanol

4,6-benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol (200 mg.) was dissolved in dioxan (3 ml.) and sodium methoxide 2.6N in methanol (0.6 ml.) was added. On standing at room temperature for less than one hour, crystals began to appear. The mixture was allowed to react overnight, the crystalline solid (water-soluble, and presumably sodium tosylate) was filtered off, and the solution concentrated under reduced pressure, and at 30° to low volume. The residue was then mixed with chloroform (10 ml.), and washed with portions of distilled water(3 x 20 ml.), and the chloroform layer dried over anhydrous sodium sulphate. Removal of the chloroform gave a white crystalline solid whose infra-red spectrum (Nujol mull) showed tosylate ester and hydroxyl to be absent. The production of this material, almost certainly the 2,3-anhydro compound, indicated that smooth cyclisation had occurred, but no further attempts at identification were made.

4,6-O-ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol

"Transethylidenation" of the benzylidene analogue (180 mg.) gave a product which, on three crystallisations from chloroform/petrol ether, gave 4,6-O-ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol (95 mg.) in white needles, m.p. 174°

(B) KINETIC PROCEDURE

Rate constants for simple bimolecular reactions are often conveniently measured in a large excess of one of the reactants, first-order kinetics being obtained. The second-order rate constant can then be calculated by dividing the first-order value by the concentration of the reactant present in excess. Baker[34] and Inglis[35], applying this principle to the cyclisation of 6-O-tosyl glycosides, examined the reactions in a large excess (at least 20-fold) of sodium hydroxide. They showed that conversion of the tosyl ester chromophore to tosylate anion is accompanied by an appreciable drop in the optical density at 265mμ, this wavelength corresponding approximately to an absorption maximum and minimum in the U.V. spectra of ester and ion respectively. Hence, assuming adherence to Beer's Law, the formation of 3,6-anhydro compounds could be followed by observing the fall in optical density associated with the release of tosylate ion.

The method used for measuring the rates of cyclisation of 4,6-O-ethylidene tosyl esters was essentially the same, 1:1 (v/v) aqueous dioxan being employed as solvent because of the limited solubility of 4,6-O-ethylidene tosylates in pure water. Changes in the optical density of 0.001M solutions of the compounds in excess sodium hydroxide (generally 0.1N) were measured at 265mμ., the use of 1cm. silica cells giving a total change in optical density of about 0.5 units. Readings were taken at fixed intervals over ca. 4-half-lives of each reaction, and a final reading ("end-value") at 10-12 half-lives, after which time the reaction was complete within the limits of detection of the instrument.

The data were then plotted according to the first-order rate equation as derived for photometric rate measurements, viz:

$$kt = 2.303 \log_{10} \frac{E_0 - E_\infty}{E_t - E_\infty}$$ where $E_0$, $E_\infty$ are the initial and final

optical densities, and $E_t$ the value after time t[20]. Under the experimental conditions which were finally developed, data were obtained for each of the reactions studied which gave good linear relationships between $-\log_{10}(E_t - E_\infty)$ and t.

Although the end-values obtained were stable for relatively long periods, the data were also processed according to the equation derived by Swinbourne[63].

$$E_t = E_\infty (1 - e^{k\Delta t}) + E_{t+\Delta t} e^{k\Delta t}$$

where $E_t$, $E_{t+\Delta t}$ and $E_\infty$ are the optical densities at times t, $t+\Delta t$ and $\infty$, and $\Delta t$ is a constant time interval, usually between 0.5- and 1- half-lives.

Thus, a plot of $E_t$ against $E_{t+\Delta t}$ gives a straight line of gradient $e^{k\Delta t}$, so that the first-order rate constant k is given by $k = \frac{1}{\Delta t} \log_e$ (slope).

This method, which gives first-order rate constants biased toward points obtained in the first stages of the reaction, does not require knowledge of the experimental $E_\infty$ (in fact an end-value can be predicted), and so provided a useful check on the rate constants calculated by the classical method. A value of $\Delta t$ equal to about one half-life was found to give the most consistent results for these experiments.

The reaction products.

From the large body of experimental information available on the reactions in alkali of the secondary tosylates of 4,6-O-acetal derivatives and allied compounds, there is no evidence to suggest that primary reactions other than cyclisation are likely to occur under the conditions reported above. Elimination reactions, involving the production of carbon-carbon double bonds, would certainly have been observed through the formation of species absorbing in the ultra-violet region. The attainment of stable end-values, corresponding to the formation of tosylate ion only, precludes the occurrence of such side-reactions. Tosylate

displacement with retention of configuration has been shown to occur under alkaline conditions in a few cases where direct cyclisation is impossible (e.g. in the initial step of the cyclisation of trans-vicinal ditosylates[40]). While there seems little likelihood of such displacements occurring in the systems investigated for the present work, the presence or otherwise of detosylated material in the reaction products could readily be confirmed by thin-layer chromatography on silica gel.

(D) Results and discussion

Preliminary experiments showed that the compounds fell into two classes which differed widely in reactivity. Methyl 4,6-O-ethylidene-2-O-tosyl-α- and -β-D-glucoside were found to be less reactive, by a large factor, than the other four compounds, and were conveniently studied at 60°, the rates of reaction with 0.1N NaOH in 1:1 aqueous dioxan being readily measurable at this temperature. For reasons discussed later, methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside was also examined in 0.02N NaOH, the ionic strength being made up to 0.1M with sodium chloride.

Methyl 4,6-O-ethylidene-3-O-tosyl-α- and -β-D-glucosides, and 4,6-O-ethylidene-2-O-tosyl- and -3-O-tosyl-1,5-anhydro-D-glucitol displayed suitable reactivities at 25° in 1:1 aqueous dioxan 0.1N with respect to sodium hydroxide. In view of the good agreement obtained among rate constants calculated by Baker and Inglis from data derived by a similar procedure, agreement between the rate constants from two runs per compound at each sodium hydroxide concentration, as calculated by the end-value and Swinbourne methods, was considered satisfactory in this work. Agreement between rate-constants derived by the two procedures, as well as between runs, was in most cases better than 2%, at worst, better than 5%.

The results are quoted in Tables 1 and 2.

TABLE 1

The rates of reaction of methyl 4,6-O-ethylidene-2-O-tosyl-α- and -β-D-glucoside with sodium hydroxide in 1:1 aqueous dioxan at 59.99 ± 0.03°

| Compound | Conc'n of compound | Conc'n of NaOH | Conc'n of NaCl | $10^4 k$ * | $10^4 k_S$ 7 |
|---|---|---|---|---|---|
| Methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside | 0.001M | 0.1N | 0 | 6.13 | 6.18 |
| " | " | " | " | 6.21 | 6.33 |
| " | " | 0.02N | 0.08M | 1.27 | 1.27 |
| " | " | " | " | 1.28 | 1.26 |
| Methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside | " | 0.1N | 0 | 18.3 | 18.4 |
| " | " | " | " | 19.0 | 18.5 |

* k = k (end-value method)

7 $k_S$ = k (Swinbourne method)

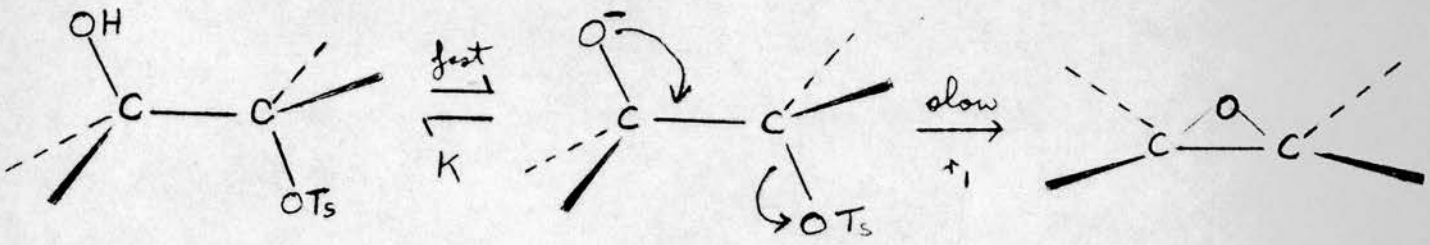
TABLE 2

The rates of reaction of methyl 4,6-O-ethylidene-3-O-tosyl-α- and -β-D-glucoside, and of 4,6-O-ethylidene-2-O-tosyl- and -3-O-tosyl-1,5-anhydro-D-glucitol with sodium hydroxide in 1:1 aqueous dioxan at 24.98 ± 0.03°

| | Conc'n of compound | Conc'n of NaOH | $10^4 k$ | $10^4 k_s$ |
|---|---|---|---|---|
| Methyl 4,6-O-ethylidene-3-O-tosyl-α-D-glucoside | 0.001M | 0.1N | 10.4 | 10.2 |
| " | " | " | 10.3 | 10.0 |
| Methyl 4,6-O-ethylidene-3-O-tosyl-β-D-glucoside | " | " | 12.1 | 12.1 |
| " | " | " | 11.8 | 12.0 |
| 4,6-O-ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol | " | " | 10.1 | 10.1 |
| " | " | " | 10.4 | 9.9 |
| 4,6-O-ethylidene-2-O-tosyl-1,5-anhydro-D-glucitol | " | " | 49.0 | 49.3 |
| " | " | " | 48.1 | 48.3 |

Before discussing the reasons for the rate differences between the compounds, the mechanism of the elementary reaction must be considered.

The cyclisation of ethylene chlorohydrin to ethylene oxide in the presence of alkali has been studied in detail[20,64], and by analogy with this reaction, the present scheme may be expressed as




Let $a$ = concentration of unionised tosylate

$a^-$ = concentration of ionised tosylate

$A$ = total concentration of tosylate = $a^- + a$

$K$ = equilibrium constant of the first step

$= \frac{a^-}{a\,[OH^-]} = \frac{K_a}{K_w}$ where $K_a$ is the ionisation constant of the tosylate, and $K_w$ is the ionic product of water

$r_1$ = first order rate constant of the second step.

Then $\frac{dA}{dt} = r_1 a^- = r_1 K [OH^-] a$

If it is assumed that the proportion of the tosylate ionised is small, then $a = A$, and

$$\frac{dA}{dt} = r_1 K [OH^-] A$$

If $a^-$ is appreciable, then

$$A = a + a^- = a + a K [OH^-]$$

Hence $a = \frac{A}{1 + K [OH^-]}$

and $\frac{dA}{dt} = r_1 K[OH^-] a = \frac{r_1 K[OH^-] A}{1 + K[OH^-]}$

Thus, in the presence of a large excess of sodium hydroxide ( $[OH^-]$ = constant), the reaction will be first-order with a rate constant ($k_1$) given by

$$k_1 = \frac{r_1 K[OH^-]}{1 + K[OH^-]} \quad \text{------} \quad (1)$$

In fact, excellent first-order behaviour was found for all the compounds studied in the present work, and also for the cyclisations of 6-tosylates studied previously.

If the proportion of ionised tosylate is very small, a can be equated to A, and the above expression reduces to

$$k_1 = r_1 K [OH^-] \quad \text{------} \quad (2)$$

This simplified expression appears to be adequate for methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside since the results in 0.1N and 0.02N sodium hydroxide show that $k_1$ is, in this case, proportional to $[OH^-]$ within the limits of experimental error (i.e. $K[OH^-]$ is negligible compared with 1.)

Since the hydroxyl at position 2 of a glycoside is generally considered to be the most acidic (c.f. p. 13 ), it might be expected that K would be larger for the glycoside 3-tosylates than for the corresponding 2-tosylates. The 3-tosylates might therefore be expected to show a non-linear dependence of $k_1$ on $[OH^-]$ (expression 2 above). Unfortunately lack of time precluded the investigation of this point. It may be noted that Baker[34] found that, for certain 6-tosylates, the term in the denominator of expression 1 was significant, but in his case, the situation was further complicated by the fact that part of the reaction proceeded via a dianion. (This is impossible for the series of compounds studied in the present work, since these contain only one hydroxyl group).

According to expression 1, differences in reactivity between different compounds may arise both from differences in K, the reaction being facilitated by an increase in acidity of the hydroxyl grouping, and also from differences in $r_1$, i.e. in the rate of cyclisation of the anion. Differences in $r_1$ may in turn arise from two sources:

(i) electronic effects affecting the ease of departure of the tosylate ion[65] and

(ii) steric effects involved in the conversion of the most stable conformation of the ion into the transition state[5]. These effects will now be considered in turn.

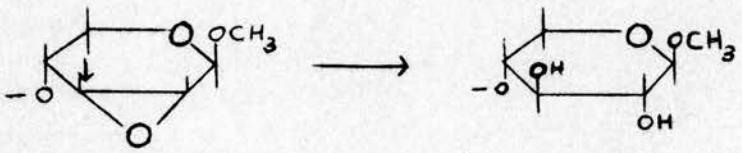
1) The influence of electronic effects on the acidity of the hydroxyl group

It is generally considered that, owing to the electron-attracting effect of the glycosidic function, a hydroxyl group at position-2 of a glycoside is more acidic than one at position-3, and this is supported by the greater reactivity of the 2-hydroxyl in reactions involving ionisation (e.g. methylation)[32]. This factor would be expected to increase the reactivity of the glycoside 3-tosylates relative to that of the glycoside 2-tosylates, and the dramatic difference in reactivity actually observed is undoubtedly due, at least in part, to this factor. Replacement of the glycosidic methoxyl group by hydrogen would be expected to decrease the acidity of a hydroxyl group at carbon-2, and hence the reactivity of 4,6-O-ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol relative to that of the corresponding glycosides. In fact, the 1,5-anhydro-glucitol-3-tosylate is of the same order of reactivity as the corresponding glucosides, the inference being that the deceleration effect associated with the lower acidity of the former compound is almost exactly counterbalanced by elimination of the steric retardations present in the glycosides.

2) Electronic effects affecting the ease of departure of the tosylate ion

There is evidence that bimolecular displacements of tosylate esters have considerable "$S_N1$ character", i.e. in the transition state, bond making has proceeded to a smaller extent than bond breaking; consequently the carbon atom tends to become positive in the transition state[65]. The displacement of tosylate would therefore tend to be retarded by electron-attracting substituents. This is found in practice, the effect being illustrated by the displacement of tosylate from n-propyl and β-ethoxyethyl tosylate by iodide ion[66]. From a purely steric point of view, the reactivities would be expected to be similar, but in fact 49.5% and 14.5% of sodium tosylate respectively, were formed when the esters were treated with sodium iodide under standard conditions.

An analogous situation arises during the epoxide ring opening of methyl 2,3-anhydro-α-D-ribosides and -α-D-lyxosides under alkaline conditions[67]. Contrary to conformational expectations, attack tends to occur at carbon-3, 2,3-anhydro-ribosides, for example, giving rise to compounds with the D-xylo configuration:



2,3-anhydro-riboside xyloside

Since alkaline ring-opening of epoxides is believed to occur by a similar mechanism to that of tosylate displacement, a possible explanation for this phenomenon is provided if the electron-withdrawing properties of the glycoside function inhibit the breaking of the carbon-2-epoxide-oxygen bond.

From the above effect, it might be expected that glycoside-2-tosylates should cyclise more slowly than their 3-O-tosyl isomers, in which the tosylate group is

more remote from the electron-attracting glycosidic group. It is clear that this electronic effect acts in the same sense as the effect, discussed above, influencing the acidities of the hydroxyl groups, and together these effects suffice to explain the dramatic differences in reactivity of the glycoside-2-tosylates as compared with their 3-tosylates.

Replacement of the methoxyl group of the methyl glucoside-2-tosylates by hydrogen will, according to the above views, tend to facilitate the displacement of the tosylate group. The greater reactivity of 4,6-O-ethylidene-2-O-tosyl-1,5-anhydro-D-glucitol as compared with the corresponding glycosides may thus be attributed, to some extent at least, to this electronic effect, though steric influences may contribute.

It may be noted that the major differences in reactivity observed in the present work (i.e. between 2- and 3-tosylates, and between the 2-O-tosyl-glucosides and -1,5-anhydro-compound) can be satisfactorily explained in terms of electronic effects. However, a discussion of the steric effects which might be involved is clearly of interest, particularly in view of Newth's suggestion that the low reactivity of methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside is due to a novel type of steric effect, namely "passing interactions"[33].

3) Steric effects

In a discussion of the steric influences affecting the reactivities of the above systems, two effects must be considered:

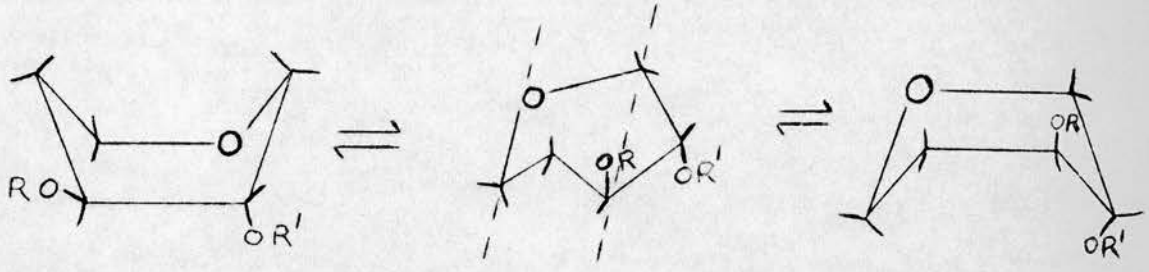
(i) effects due to differences in steric compression between the stable conformation of the tosylate and the transition state of the reaction, and

(ii) passing interactions.

These will be considered in turn.

(i) Effects due to differences in steric compression between ground and transition states of the tosylate

Epoxide formation is one of many reactions which has the stereochemical requirement that the four centres involved in the reaction (in this case $O_2$, $C_2$, $C_3$, and $O_3$) must lie, at least approximately, in one plane[5]. This is equivalent to the requirement that the dihedral angle between $C_2$-$O_2$ and $C_3$-$O_3$ should be near 180°. Since the dihedral angle in the Cl conformation of the glucosides is ca. 60°, it is clear that a change in conformation must occur before reaction can take place. Remembering the restrictions imposed by the trans-fused acetal ring, it is clear that the only possible reactive conformations are 2B (dihedral angle 180°), Bl (dihedral angle 120°) and the intermediate skew forms (dihedral angle between 120 and 180°).

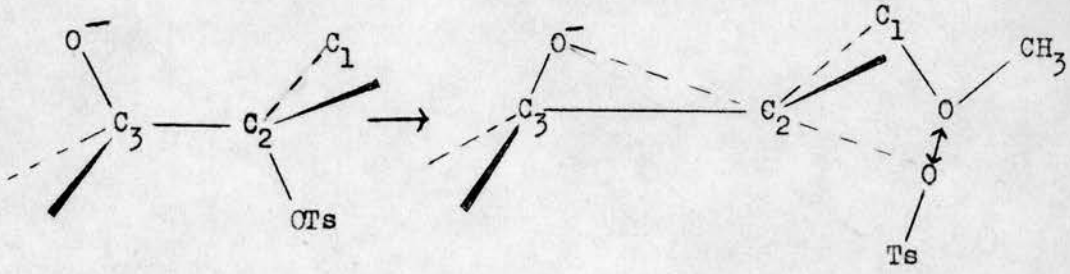



Bl Midway skew 2B

where R ≠ $R^1$ = H or Ts

The actual transition state may be expected to have a conformation intermediate between one of the above and the half-chair conformation of the final epoxide. Since the exact conformation of the transition state is uncertain, a precise discussion of the steric factors is not possible, but the following qualitative observations seem relevant.

Examination of Dreiding Models* suggests that the reactive conformations (B1, 2B and intermediate skews) might be expected to be somewhat less favoured for β-anomers; for these, there are unfavourable axial-axial interactions between $O_1$ and $O_3$ in the 2B conformation (interatomic distance ca. 2.6Å), and "bow and stern flagpole" interactions between $O_1$ and $H_4$ in the B1 conformation (interatomic distance ca. 1.7 Å). These interactions would be present, though to a lesser degree, in the intermediate skew forms. The fact that the 2-O-tosyl-β-glucoside is about three times as reactive as the corresponding α-anomer is thus not anticipated on steric grounds. While it is possible that dipole interactions between $O_1$ and the ring oxygen, which are known to favour the change from an equatorial to an axial anomeric group, are acting in opposition, and swamping the purely steric repulsions described above, these do not account for the difference between the α/β ratios obtained for the glucoside 2- and 3- tosylates.[7] An additional factor must be involved and it is tentatively suggested that the lower relative reactivity of the α-glucoside-2-tosylate may be related to the stereochemistry of the transition state as follows.




* Steel models with bonds constructed to scale, and free single-bond rotation.

7 NOTE: Both steric and dipole interactions appear to be reduced as the molecule approaches the final transition state.

If $O_3^-$, $C_2$ and $O_2$ are to become approximately collinear (the basic reason for the trans-planar requirement[5]) then, as can be seen by examination of models, $O_2$ (in the departing tosylate group) must approach $O_1$ of the α-glycoside so as to become almost eclipsed with it. The effect may be enhanced by the lengthening of the $C_2 - OT_S$ bond.

The interpretation of the relative reactivities of corresponding anomers may thus be summarised as follows:-

3-tosylates The similar reactivity of these anomers is explained if the retardation of the α-tosylate associated with dipole-derived stability of the axial anomeric methoxyl is approximately equal to the net retardation of the β-tosylate due to steric retardation as already described, together with some accelerating influence arising from dipole repulsions encountered by the equatorial anomeric methoxyl.

2-tosylates Similar arguments apply to the 2-tosylates, the α-anomer being further retarded by the above-mentioned $O_2 - O_1$ interference.

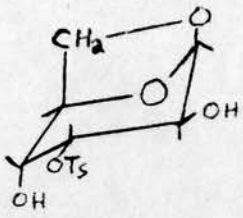
On replacement of the anomeric methoxyl by hydrogen, the above effects are eliminated. In terms of steric considerations, therefore, 1,5-anhydro-D-glucitol derivatives would be expected to react faster than the corresponding glycosides, and for the 2-tosylates this is so, although in this case, electronic effects probably contribute (c.f. p. 54 ). As previously mentioned, however (p. 53 ), 4,6-O-ethylidene-3-O-tosyl-1,5-anhydro-D-glucitol is of similar reactivity to the corresponding glycosides, and here, the steric and electronic effects of methoxyl substitutions probably act in opposition, the net result being a mutual cancellation.

No likely explanations for the wide difference in reactivity (a factor of 5) between the 1,5-anhydro-glucitol-2- and -3-tosylates have as yet been offered. The reasons are probably steric in origin, since the electronic environments of

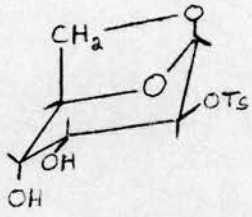
hydroxyls 2 and 3 are very similar.

(ii) Passing Interactions

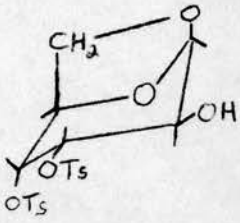
This type of steric effect was first invoked by Newth to explain striking differences in the ease of formation of epoxides from certain secondary tosylates[33]. In his review on sugar anhydrides, he refers to his own work in which the 2- and 3-monotosylates, and the 3,4-ditosylate of 1,6-anhydro-β-D-altrose(formulae **VIII,VII & IX** respectively), were treated with alkali under the same experimental conditions[68].




VII




VIII




IX

The 3-tosylate was found to be smoothly converted to the 2,3-anhydro-mannoside, though at a slow rate, while the 2-tosylate and 3,4-ditosylate were resistant to alkaline attack. Assuming that the tosylate group is "bulkier" in the conformational sense than the hydroxyl (not necessarily true, as can be seen from models; see also ref. 5b), Newth interpreted these differences in reactivity from a purely steric standpoint. On moving from the stable 1C conformation to the "reactive" conformation (in this case taken to be the boat 3B), the cis-oriented substituents on carbons 3 and 4 must pass one another, and the resulting interaction is said to retard the cyclisation at a rate-determining stage. These passing interactions, in conjunction with the steric interactions experienced by axial-axial and eclipsed substituents in the transition state, are claimed to determine whether or not the reactions will proceed, i.e. to determine the relative rates of cyclisation of the

tosylates.

By these arguments, compound **VII** above will have only one serious rate-retarding influence, an $OTs_3/OH_4$ passing interaction; the cyclisation will therefore proceed at a slow rate. Compound **VIII**, the 2-tosylate, will have in addition to an $OH_3/OH_4$ passing interaction, serious $OTs_2/O_1$ and $OTs_2/C_6$ cross-ring interactions in the transition state, sufficient, it would seem, to prevent reaction. Cyclisation of the 3,4-ditosylate, compound **IX** would be inhibited largely by the $OTs_3/OTs_4$ passing interaction.

The same type of argument was used by Newth to explain the very large difference in reactivity between methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside and 4,6-O-benzylidene-2-O-tosyl-1,5-anhydro-D-glucitol, (apparent from preparative work, and confirmed in the present kinetic study for the corresponding 4,6-O-ethylidene derivatives). Thus, there would be a passing interaction between the α-glycoside methoxyl group and the 2-tosylate group, this inhibitory influence being absent for the 1-deoxy analogue. However, the present work shows that the corresponding β- anomer methyl 4,6-O-ethylidene-2-O-tosyl-β-D-glucoside, in which passing interactions are also absent, is only slightly (a factor of 3) more reactive than the α-glycoside, and the very large difference in reactivity between the 1,5-anhydro-D-glucitol-2-tosylate and the glucoside-2-tosylates cannot therefore be ascribed to passing interactions, but must be due to the electronic effect discussed in page 54 (i.e. that tosylate is more readily displaced when the adjacent electron attracting methoxyl is replaced by hydrogen).

Further, it seems likely that the differences in reactivity among the 1,6-anhydro-altrose tosylates discussed by Newth should also be ascribed to electronic effects. The differences in reactivity between the 2- and 3-monotosylates can thus be explained electronically in terms of the effects (1) and (2) discussed earlier (c.f. the

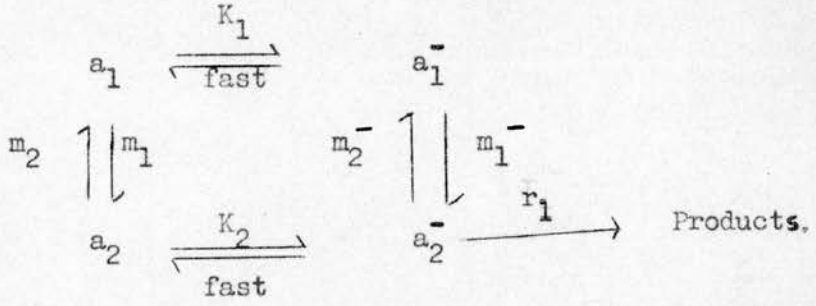
difference between the 2- and 3-tosylates studied in the present work). The low reactivity of the 3,4-ditosylate can again be explained electronically, since the electron-attracting tosylate group on $C_4$ would tend to retard the displacement of the 3-tosylate (effect (2) ). It is therefore apparent that the concept of passing interactions is subject to considerable doubt.

Another observation which has been interpreted by Newth in terms of passing interactions is the much greater reactivity of methyl 4,6-O-benzylidene-2-O-tosyl-β-D-galactoside as compared with its α-anomer. This case is not entirely unambiguous since, in contrast with the glucoside 4,6-acetals, the cis-fused ring makes the system relatively mobile, and further the experimental evidence is not above doubt. Wiggins[69] reported that the α-anomer did not react with sodium methoxide in chloroform-methanol in the cold, conditions under which the β-anomer reacted smoothly. However, the quantity of methanol added to the solution was not reported, and it seems likely that the basicity of such solutions will be very sensitive to the quantity of methanol present. Also, as reported in the present work (page 44 ), alkaline mixtures containing chloroform are somewhat unsatisfactory reagents because of reaction of the alkali with chloroform. A further unsatisfactory feature is the striking discrepancy between the observations of Wiggins[69] and Reber and Reichstein[58] regarding the reactivity of the α-anomer with sodium methoxide in boiling methanol (examination of the literature shows that there is an approximately tenfold difference in the concentrations of sodium methoxide used, but even this seems insufficient to resolve the contradiction). It is clear that a quantitative examination of the galactoside tosylates would be of interest.

Although the preceding discussion throws considerable doubt on the importance of passing interactions, it is necessary to consider whether the three-fold difference in rate found between methyl 4,6-O-ethylidene-2-O-tosyl-α-and -β-D-glucosides could

be ascribed to the retardation of the reaction of the α-anomer by passing interactions.

"Passing interactions" must be regarded as energy barriers between alternative conformations, and if such barriers are to affect the rate of a reaction, then the interconversion must be slow, or at least of similar rate, as compared with the other steps. This is contrary to the assumption usually made that the interconversion of conformations is fast compared with the rate-determining step of reactions[5]. In fact, the kinetic equations derived earlier (equations 1 and 2, p. 52) depend upon this assumption and, if passing interactions are kinetically significant, the reaction scheme given earlier is inadequate. The following scheme, in which $a_1$ and $a_2$ represent the stable and reactive conformation respectively, may be used as a basis for the kinetic discussion of passing interactions.




The rigorous derivation of a rate equation from the above scheme appears to be difficult, so the following simplifying assumptions will be made:

(a) that $K_1 = K_2$

(b) that $m_1 = m_1^-$ and $m_2 = m_2^-$

(c) that the stationary state hypothesis can be applied to $a_2 + a_2^-$ (the concentrations of unstable conformers) and

(d) that the total concentration (A) of tosylate = $a_1 + a_1^-$ i.e. that the concentration of the unstable conformer is very small.

It is recognised that (a) and (b) may not be strictly true, but this should not alter the essential features of the conclusions. The approximate truth of assumptions (a) and (b) may be justified by the consideration that it seems unlikely that the reaction would proceed almost entirely by one of the two pathways only at the expense of the other.

Applying assumptions (a) and (b), the scheme reduces to

$$a_1 \underset{\text{fast}}{\overset{K_1}{\rightleftharpoons}} a_1^-$$

$$m_2 \downarrow \uparrow m_1 \qquad m_2 \downarrow \uparrow m_1$$

$$a_2 \underset{\text{fast}}{\overset{K_1}{\rightleftharpoons}} a_2^- \xrightarrow{r_1} \text{Products}$$

Then, applying assumption (c):

Rate of formation of $(a_2 + a_2^-)$ = rate of removal of $(a_2 + a_2^-)$,

$$\therefore \quad a_1^- m_1 + a_1 m_1 = a_2 m_2 + a_2^- m_2 + a_2^- r_1$$

Now $a_1^- = a_1 K_1 \left[OH^-\right]$ and $a_2^- = a_2 K_1 \left[OH^-\right]$

Substituting for $a_1^-$ and $a_2$ in the first equation, we have

$$a_1 m_1 K_1 \left[OH^-\right] + a_1 m_1 = \frac{a_2^- m_2}{K_1 \left[OH^-\right]} + a_2^- m_2 + a_2^- r_1$$

$$\therefore \quad a_2^- = \frac{a_1 m_1 \left(1 + K_1 \left[OH^-\right]\right)}{r_1 + m_2 + m_2 / K_1 \left[OH^-\right]} = \frac{a_1 m_1 K_1 \left[OH^-\right] \left(1 + K_1 \left[OH^-\right]\right)}{m_2 + (r_1 + m_2) K_1 \left[OH^-\right]}$$

Thus, the all-over rate of reaction is $\frac{dA}{dt} = r_1 a_2^-$

$$= \frac{r_1 a_1 m_1 K_1 \left[OH^-\right] \left(1 + K_1 \left[OH^-\right]\right)}{m_2 + (r_1 + m_2) K_1 \left[OH^-\right]}$$

$$= \frac{A r_1 m_1 K_1 \left[OH^-\right]}{m_2 + (r_1 + m_2) K_1 \left[OH^-\right]}$$

since $A = a_1 + a_1^- = a_1 + a_1 K \left[OH^-\right]$

$$= a_1 \left(1 + K_1 \left[OH^-\right]\right)$$

Hence, in the presence of a constant excess of sodium hydroxide, the

reaction will be first-order with a rate constant given by

$$k_1 = \frac{r_1 m_1 K_1 [OH^-]}{m_2 + (r_1 + m_2) K_1 [OH^-]}$$

$$= \frac{r_1 m_1/m_2 K_1 [OH^-]}{1 + K_1 [OH^-] + r_1 K_1 [OH^-]/m_2} \qquad (3)$$

In the absence of significant passing interactions, the two conformations would always be in equilibrium, so that

$$a_2^- = \frac{m_1}{m_2} a_1^- \text{ , and under these conditions}$$

$$\frac{dA}{dt} = r_1 a_2^- = r_1 \frac{m_1}{m_2} a_1^- = r_1 \frac{m_1}{m_2} a_1 K_1 [OH^-] = \frac{A r_1 m_1/m_2 K_1 [OH^-]}{1 + K_1 [OH^-]}$$

The first-order rate constant would then be

$$k_1 = \frac{r_1 m_1/m_2 K_1 [OH^-]}{1 + K_1 [OH^-]} \qquad (4)$$

It may be noted that 3 reduces to 4 when $m_2 >> r_1$.

Now, if the three-fold difference between the first-order rate constants of methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside ($k_1^\alpha$) and the corresponding β-anomer ($k_1^\beta$) in 0.1-N NaOH is to be ascribed to the retarding influence of passing interactions on the former compound, then, to a close approximation

$$k_1^\alpha = 1/3\, k_1^\beta$$

$$\text{i.e.} \quad \frac{1}{1 + 0.1K_1 + 0.1\, r_1/m_2\, K_1} = 1/3 \times \frac{1}{1 + 0.1K_1}$$

$$\therefore \quad 0.1 \frac{r_1}{m_2} K_1 = 2 + 0.2K_1 = \text{at least } 2$$

$$\therefore \quad \frac{r_1}{m_2} K_1 = \text{at least } 20$$

It is clear that a term of this magnitude in the denominator of equation 3 should be detectable by examining the dependence of $k_1$ upon $[OH^-]$ for the α-anomer. In the presence of passing interaction the derived second-order rate constant $\frac{k_1}{[OH^-]}$ is related to the hydroxide concentration by the expression

$$\frac{k_1}{[OH^-]} = \frac{r_1 m_1/m_2 K_1}{1 + K_1 [OH^-] + r_1/m_2 K_1 [OH^-]}$$

Then, if $\frac{r_1}{m_2} K_1 = 20$, a reduction in hydroxyl ion concentration from 0.1N to 0.02N should increase $\frac{k_1}{[OH^-]}$ by a factor of at least $\frac{1 + 2}{1 + 0.4} \simeq 2$. The first-order rate constant ($k_1$) for the α-anomer was therefore determined at the latter concentration, the ionic strength being adjusted to 0.1M with sodium chloride to reduce complications due to differences in this factor. The results show that within experimental error (about 3 %), $\frac{k_1}{[OH^-]}$ did not vary. It therefore seems unlikely that the difference in reactivity between the glucoside-2-tosylate anomers can be ascribed to passing interactions.

The conclusion reached from the above theoretical analysis, that significant passing interactions should lead to an increase in $\frac{k_1}{[OH^-]}$ when the hydroxide concentration decreases, seems plausible on more general grounds. When the rate of formation of products is slow (as at low $[OH^-]$), more time is available for the attainment of conformational equilibrium; the effect of passing interactions should therefore be less significant than at higher concentrations.

An extension of this work would be of interest, but some difficulties would undoubtedly be encountered. In the altroside series, extremely mild conditions would have to be developed to follow the reactions of these highly reactive compounds[70]. The relative reactivities of the galactoside tosylates in alkali would also be of interest, as mentioned above, and study of the corresponding

idosides would complete this series of 4,6-O-acetal derivatives. Evaluation of the steric changes in the last two groups of compounds would, however, be even more complicated since they contain the more flexible cis-fused ring system.

# EXPERIMENTAL

## Description of Apparatus

Kinetic measurements were made spectroscopically according to the procedure outlined on pages 45 - 46 using a Unicam SP.500 spectrophotometer. For the lower temperature experiments, the instrument was fitted with a Unicam SP.570 constant temperature cell housing, water from a bath thermostatted at 25.01 ± 0.03° being circulated through the cell holder by means of a Stuart Turner No.10 pump fitted in the return stream from the cell housing. The water temperature, which was controlled using a Shandon Circotherm thermostat unit in conjunction with copper cooling coils, fell to a lower equilibrium value in the cell housing due to the lower ambient temperature (20° ± 1°); this cooling was partly compensated by the heating effect of the adjacent hydrogen lamp, the cell temperature under experimental conditions being 24.97 ± 0.03°.

During preliminary experiments, similar apparatus was used to provide thermostatting for the runs performed at 60°, Diala B oil being substituted for water, and both temperature control and circulation through the cell holder being obtained by means of the commercial "Tempunit" (Techne, Cambridge). However, the temperature fluctuations of the cell housing observed using the above arrangement proved unacceptable, and the Unicam SP.570 housing was replaced by the Adkins Thermostatted Cell Holder. In this latter apparatus, the temperature of the cell block was controlled by means of a heating-coil and thermistor, a temperature of 59.99 ± 0.03° being retained in the reaction cell for long periods. Corrections for a slow temperature drift, which occurred periodically, were readily applied using an external variable resistance in the circuit of the Control Unit. For the work at 60°, the temperature control of the oil/Tempunit bath proved

satisfactory to permit its use in preheating reactant solutions and syringes, and as a constant-temperature "hot-junction" for the thermocouple work described later.

All thermometers used were calibrated against a corresponding N.P.L. standard, further corrections being applied at the higher temperatures for the exposed stem. For measuring rapid temperature fluctuations, a thermocouple was constructed from high-gauge thermocouple wire, the junctions being arc-welded. Small changes in e.m.f. were measured using a suitable low resistance "Scalamp" galvanometer. A sensitivity of 1.5 cm. per °C temperature difference was achieved with this apparatus.

Preparation of Reactant Solutions

Resin-deionised water and "Spectroscopic Grade" dioxan commercially available from B.D.H. were used in all the kinetic work. In some preliminary experiments, the dioxan provided was found to react with sodium hydroxide solution to give small amounts of a species absorbing at 265mμ. The material was destroyed on standing, the absorption of an alkaline solution of aqueous dioxan at 60° falling off rapidly to a low constant value. This interfering compound was readily removed from the dioxan prior to use by pretreatment of the liquid with solid sodium hydroxide for 24 hours at room temperature, the solid being removed by rapid filtration through a sintered glass funnel.

To minimise errors arising from the volume changes associated with mixing dioxan with water, 0.002M solutions of the tosylates were prepared from freshly-made aqueous dioxan, the solvent mixture consisting of equal volumes of dioxan and deionised water. Aqueous sodium hydroxide solutions were prepared as required by appropriate dilutions of N/1 or N/10 standard solutions made from B.D.H. concentrated volumetric solutions. These solutions, as supplied, were shown by Baker[34] and

Inglis[35] to be substantially free from carbonate. The stock standards were rejected after a week or so, fresh solutions being prepared. Where adjustments in ionic strength were required, the appropriate amounts of "Analar" sodium chloride were introduced during the dilution of the stock solutions.

Procedure immediately prior to kinetic measurements

At 25° The glass reaction cell consisted of a two-compartment H-shaped vessel, the hollow bridge permitting reactant solutions to be readily mixed; the two necks were fitted with Quickfit sockets provided with the appropriate stoppers. 10 ml. of the tosylate solution was pipetted into one arm of the reaction vessel, and the air flushed out with carbon dioxide-free nitrogen. 5 ml. of dioxan and 5 ml. of aqueous sodium hydroxide of the appropriate concentration (0.4N in most cases) were then delivered into the other arm, and both compartments stoppered. To prevent the formation of interfering air-bubbles during spectrophotometric measurements, the dissolved air was now expelled from the solutions by immersing the cell above the level of the liquids in a water-bath at about 40° for 5-10 minutes. The vessel was then immersed in the water-bath thermostatted to 25° for about 30 minutes to come to thermal equilibrium.

Meanwhile, two matched 1 cm. silica Unicam cells (with stoppers) were placed in the thermostatted cell-carrier, one of these being filled with a solution of M/1000 sodium tosylate in 1:1 aqueous dioxan preheated at 40° as described above. This solution was employed as a blank, the optical density of the reaction solution during kinetic measurements falling conveniently from about 0.5 to zero units. The temperature difference between cell and water bath (0.04°) did not affect the reaction rate at the lower temperature.

Immediately prior to kinetic measurements, the reaction vessel was removed from the water bath, and inverted several times to mix the reactant solutions

thoroughly; this was taken as zero time for the reaction. A sample of the reaction mixture of the required volume was then rapidly transferred to the empty Unicam cell by means of a graduated glass syringe, the first reading being obtainable within two minutes of mixing.

At 60° While the kinetic procedure for the less reactive group of tosylates was essentially similar to that described above, a number of precautions had to be observed, necessitated by the use of an elevated temperature. During preliminary work, variations in the absorption of the blank, attributed to evaporation, were reduced by fitting the Unicam cell stoppers with "Teflon" sleeves. Satisfactory blank stability was not, however, obtained until the Unicam cells were replaced by narrow-mouthed "Thermal Syndicate" cells, fitted with specially designed "Teflon" stoppers. Similar cells were employed for the reaction mixtures.

The cooling of the reaction solutions during mixing and transference presented a further problem. To minimise these heat losses, the reaction vessel was immersed into the oil-bath as near the stoppers as possible without risk of contamination of the reactants with oil; after mixing, the vessel was returned to the bath before transference of the reaction solution. The syringe used was also preheated by placing in a long thin-walled test-tube which was immersed well into the oil-bath. In spite of all precautions, a slight drop in temperature was unavoidable, the temperature readjustment manifesting itself by a curving of the End-value and Swinbourne plots corresponding to the first few minutes of each reaction.

To determine the conditions which gave the most rapid temperature equilibration in the Unicam cell, the transference procedure was examined using the thermocouple described on page 68 . In using the oil-bath for heating the "hot junction", the effects of local temperature variations were largely reduced by placing the junction in a small tube of oil immersed in the bath; a stability

of ± 0.05° was obtained. For convenience, the wires leading to the "cold junction" were stuck down two grooves in each side of a Unicam stopper by means of adhesive, so that the junction could be introduced into the solution, and the cell stoppered in one operation.

Due largely to the independent thermostatting of the two junctions, galvanometer readings were not stable, and fluctuations of ± 1 m.m. or more were encountered. However, the temperature increment/galvanometer change relationship was approximately linear, and for the purposes of this investigation, the system was considered adequate. Using aqueous dioxan solutions, conditions of mixing and transference of reaction mixtures were simulated exactly. In determining the best temperature for preheating reactant solutions, bath temperatures of 0.5 to 4.0° above Unicam cell temperature were used, the times for equilibration in the cell being noted. It was found that the use of higher preheating temperatures offered no advantage, the average time for a steady galvanometer reading being 6 minutes for the temperature range studied. A decrease in equilibration time obtained by reheating the mixed solutions in the oil-bath for 1-3 minutes before transference was slight.

For the kinetic experiments, a bath temperature of 60.5° was chosen, the reaction vessel being returned to the bath after mixing, and the reaction mixture transferred at once to the Unicam cell.

Kinetic measurements

As soon as transfer of the reaction solution was complete, the cell was stoppered, the compartment lid replaced, and spectroscopic measurements commenced at once. It thus became established practice, immediately prior to mixing, to check the stability of the instrument, to set the dark current to zero, and to "zero" the blank solution using the slit width control. Any inconsistencies in

the behaviour of the instrument, as well as variations in the blank due to evaporation, air-bubbles etc., could usually be observed by noting the absorption of the blank against air before and after each run.

Readings were taken at constant time intervals over at least 3-4 half-lives of each reaction, the interval being doubled when consecutive readings differed by less than 0.005 in their absorbance (after about 2 half-lives). The "zeroing" of the blank solution was checked as often as convenient during each run, and after each reading when time intervals between readings were greater than one minute. The end-value of each reaction was read off after 12-15 half-lives, these values, after elimination of variations due to dioxan impurities and evaporation, being essentially stable for all the reactions studied.

First-order rate constants were determined from each set of acceptable data by the end-value and Swinbourne methods, as described earlier. Rate constants were accepted when good agreement was obtained by both methods of calculation between two runs in which stable end-values were encountered, and which gave good linear plots over at least 3 half-lives for Swinbourne and end-value data.

Typical sets of experimental data for runs at 25° and 60° are shown in Tables 3 and 4 , the corresponding end-value and Swinbourne graphs being plotted out on pages 74 and 76.

TABLE 3

Typical experimental data

for the reaction of methyl 4,6-O-ethylidene-3-O-tosyl-β-D-glucoside

in aqueous dioxan (1:1) 0.1N with respect to sodium hydroxide at 25.01 ± 0.03°.

| Time in mins. | $E_t$ | $E_t + \Delta t^*$ | $\log_{10} \frac{1}{E_t - E_\infty}$ 7 |
|---|---|---|---|
| 0 | - | - | - |
| 2 | 0.390 | 0.215 | 0.480 |
| 3 | 0.350 | 0.2035 | 0.536 |
| 4 | 0.3325 | 0.195 | 0.563 |
| 5 | 0.317 | 0.185 | 0.588 |
| 6 | 0.2985 | 0.176 | 0.621 |
| 7 | 0.281 | 0.169 | 0.654 |
| 8 | 0.267 | 0.161 | 0.682 |
| 9 | 0.2515 | 0.154 | 0.713 |
| 10 | 0.239 | 0.148 | 0.745 |
| 11 | 0.227 | 0.1415 | 0.775 |
| 12 | 0.215 | 0.136 | 0.807 |
| 13 | 0.2035 | 0.131 | 0.840 |
| 14 | 0.195 | 0.126 | 0.867 |
| 15 | 0.185 | 0.1215 | 0.900 |
| 16 | 0.176 | 0.1175 | 0.932 |
| 17 | 0.169 | 0.114 | 0.959 |
| 18 | 0.161 | 0.110 | 0.987 |
| 19 | 0.154 | 0.106 | 1.022 |
| 20 | 0.148 | 0.103 | 1.051 |
| 21 | 0.1415 | - | 1.084 |
| 22 | 0.136 | 0.092 | 1.114 |
| 23 | 0.131 | - | 1.142 |
| 24 | 0.126 | 0.092 | 1.174 |
| 25 | 0.1215 | - | 1.204 |
| 26 | 0.1175 | 0.087 | 1.233 |
| 27 | 0.114 | - | 1.260 |
| 28 | 0.110 | 0.084 | 1.293 |
| 29 | 0.106 | - | 1.328 |
| 30 | 0.103 | 0.0805 | 1.357 |
| 32 | 0.0975 | 0.0775 | 1.415 |
| 34 | 0.092 | - | 1.481 |
| 36 | 0.087 | 0.0735 | 1.553 |
| 38 | 0.084 | - | 1.602 |
| 40 | 0.0805 | 0.070 | 1.668 |
| 42 | 0.0775 | - | 1.733 |
| 100 | 0.059 | | |
| 120 | 0.059 | | |

$\Delta t$ = 10 mins. 7 $E_\infty$ = 0.059

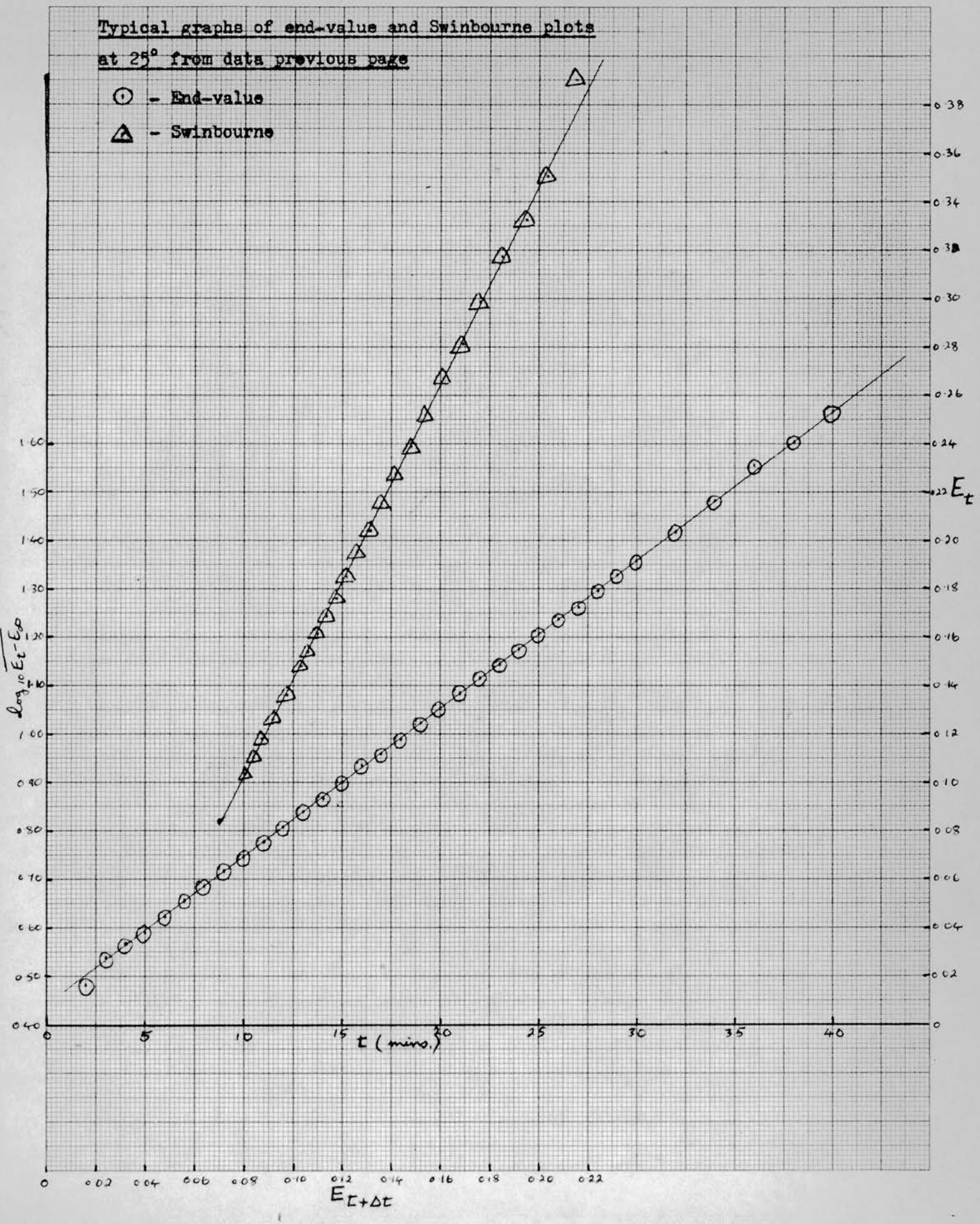

Typical graphs of end-value and Swinbourne plots
at 25° from data previous page
⊙ - End-value
△ - Swinbourne
$\frac{1}{\log_{10} E_t - E_\infty}$
$E_t$
t (mins.)
$E_{t+\Delta t}$

TABLE 4

Typical experimental data

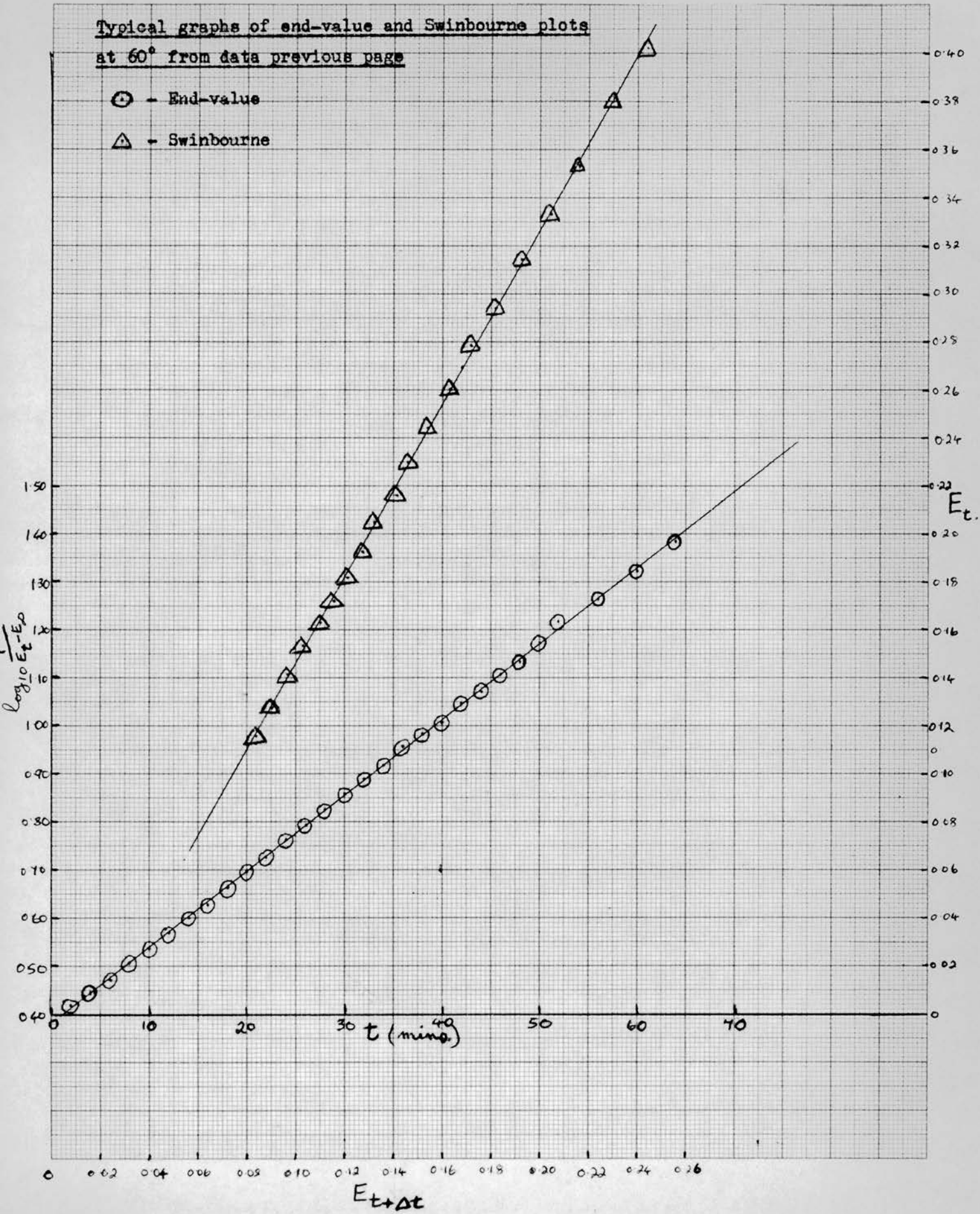
for the reaction of methyl 4,6-O-ethylidene-2-O-tosyl-α-D-glucoside

in aqueous dioxan (1:1) 0.1N with respect to sodium hydroxide at 59.99 ± 0.03°.

| Time in mins. | $E_t$ | $E_t + \Delta t$* | $\log_{10} \frac{1}{E_t \; 1 - E_\infty}$ 7 |
|---|---|---|---|
| 0 | - | - | - |
| 2 | 0.424 | 0.260 | 0.419 |
| 4 | 0.4015 | 0.244 | 0.445 |
| 6 | 0.381 | 0.230 | 0.471 |
| 8 | 0.353 | 0.216 | 0.508 |
| 10 | 0.333 | 0.2045 | 0.537 |
| 12 | 0.312 | 0.1925 | 0.569 |
| 14 | 0.2935 | 0.182 | 0.600 |
| 16 | 0.278 | 0.172 | 0.628 |
| 18 | 0.260 | 0.163 | 0.663 |
| 20 | 0.244 | 0.1535 | 0.696 |
| 22 | 0.230 | 0.146 | 0.724 |
| 24 | 0.216 | 0.141 | 0.761 |
| 26 | 0.2045 | 0.132 | 0.791 |
| 28 | 0.1925 | 0.1275 | 0.824 |
| 30 | 0.182 | 0.121 | 0.855 |
| 32 | 0.172 | 0.116 | 0.888 |
| 34 | 0.163 | 0.110 | 0.919 |
| 36 | 0.1535 | 0.103 | 0.955 |
| 38 | 0.146 | - | 0.985 |
| 40 | 0.141 | 0.097 | 1.006 |
| 42 | 0.132 | - | 1.048 |
| 44 | 0.1275 | 0.090 | 1.071 |
| 46 | 0.121 | - | 1.105 |
| 48 | 0.116 | 0.084 | 1.134 |
| 50 | 0.110 | - | 1.171 |
| 52 | 0.103 | - | 1.218 |
| 56 | 0.097 | - | 1.264 |
| 60 | 0.090 | - | 1.323 |
| 64 | 0.084 | - | 1.382 |
| 200 | 0.0425 | | |
| 260 | 0.0425 | | |

* $\Delta t$ = 16 mins.

7 $E_\infty$ = 0.0425


Typical graphs of end-value and Swinbourne plots
at 60° from data previous page
⊙ - End-value
△ - Swinbourne
$\log_{10}\frac{1}{E_t - E_\infty}$
$E_t$
t (mins)
$E_{t+\Delta t}$

# PART II

## The Aqueous Solvolysis of Glycoside 6-tosylates

### Introduction

The preliminary studies reported below were carried out to assess the potential of this topic as a long-range research project. For reasons discussed below, the difficulties involved in a detailed investigation of these solvolyses seemed too great and varied to justify an extension of the work, but the results obtained were in themselves of interest, particularly in view of the discovery of a novel neighbouring-group reaction involving an unionised hydroxyl group. The results of the experiments are discussed in the light of recent conformational theories, and the systems critically compared with the analogous reactions in alkali.

### Background

Genuine solvolysis reactions involving unionised species are relatively rare in carbohydrate chemistry[71] except for reactions at carbon-1. Methyl 4,6-O-benzylidene-2,3-anhydro-α-D-alloside, isolated by Honeyman and his co-workers[72] during the elution of the corresponding altroside 2-tosylate from a "neutral" alumina column, may have resulted from a reaction under neutral conditions, though the possibility that the alumina was incompletely neutralised cannot be discounted. Interest was therefore aroused when, in a system containing a glycoside 6-tosylate and water, slow release of tosylate was observed, even in the absence of alkali. However, recognition of the reaction as a solvolysis was somewhat delayed since in the original discovery of this phenomenon by Baker[34], the release of tosylate ion at room temperature (determined spectroscopically) was first observed in 3M sodium

chloride solution. In view of the well-known nucleophilic replacement of a primary tosylate group by iodide ion[37], an analogous chloride reaction was suspected. Reactions with stronger nucleophiles were therefore examined under aqueous conditions. Since the reaction of potassium iodide with methyl 6-O-tosyl-β-D-galactoside was complicated by release of iodine, the reaction of the strongly nucleophilic thiosulphate ion with methyl 6-O-tosyl-β-D-galactoside and methyl 6-O-tosyl-α-D-glucoside was followed spectroscopically at 273mμ. In agreement with Tipson's observations[37] on rates of desulphonyloxylation, the glucoside 6-tosylate was found to react about five times faster than the galactoside 6-tosylate, the reactivities being in the reverse order to those observed by Baker for the sodium chloride systems. It was then found that the tosylates reacted with water even in the absence of sodium chloride and at much the same relative rates, and the reaction, now considered to be predominantly a solvolysis, was studied in greater detail.

The Products of the reaction

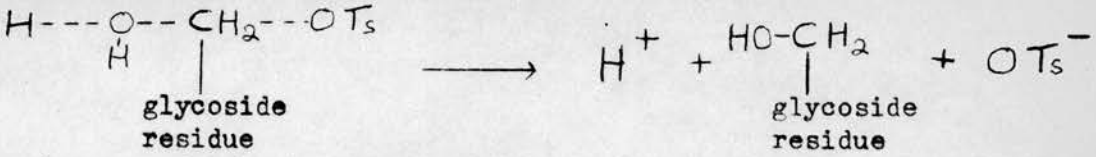
As will be seen from the kinetic results, the solvolysis reactions at room temperature of the 6-tosylates of methyl α- and β-D-glucoside and methyl α- and β-D-galactoside are extremely slow, the galactosides being, however, perceptibily more reactive than the glucosides. The most striking feature of these reactions lay in the unexpectedly high reactivity of the β-galactoside, toluene-p-sulphonic acid being released at a rate several times greater than for the α-galactoside, and many times greater than for the glucosides. Noting that this sequence of reactivities was reproduced at 50°, methyl 6-O-tosyl-β-D-galactoside was chosen for analytical studies of reaction products, the solvolysis at this temperature being essentially complete after about 5 days.

Having established that the reaction was a true solvolysis, two possible types

of mechanism were postulated for the primary reaction:

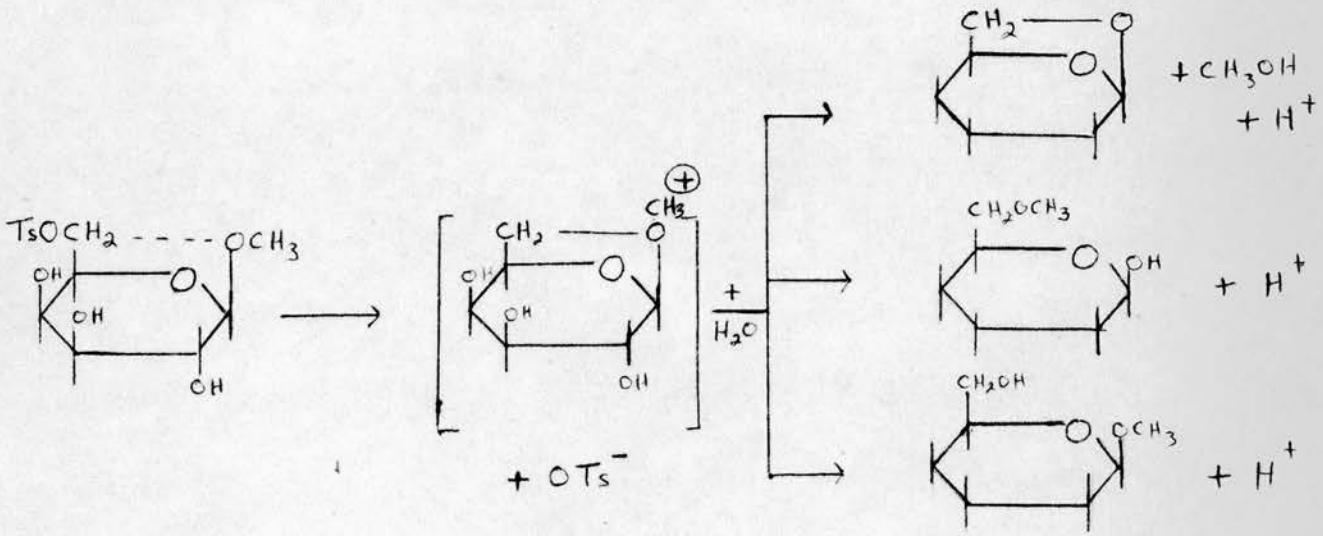
(a) $S_N2$ attack of water on carbon-6, (c.f. 73)

Expressed in its simplest form, the reaction may be written




While analogous solvolysis reactions for simple alkyl sulphonyl esters have been reported, a mechanism of this kind, in which a conformational change of the carbohydrate ring would be unnecessary (the primary $CH_2OTs$ group being equatorial), would not explain the large dependence of reaction rate on the molecular configuration at carbon-1.

(b) A neighbouring group reaction on carbon-6, involving the 3-hydroxyl, or possibly in the case of β-anomers, the anomeric methoxyl. In the former case the 3,6-anhydro-glycoside would be the sole product, while in the latter case several products could arise; for example, for the β-galactoside:



Thus, for methyl 6-O-tosyl-β-D-galactoside, the possible end-products are methyl β-D-galactoside from an $S_N2$ reaction with water and perhaps D-galactose

in the event of glycoside hydrolysis; methyl 3,6-anhydro-β-D-galactoside and 3,6-anhydro-D-galactose from 3,6 neighbouring group reaction; 1,6-anhydro-β-D-galactose, 6-O-methyl-D-galactose, methyl β-D-galactoside (c.f. water attack) and possibly D-galactose from 1,6 neighbouring group reaction. In order to determine the kind(s) of reaction occurring during solvolysis, paper chromatographic techniques were employed.

Chromatographic Analysis. Paper chromatography by the descending technique was used, the top layer of a butanol:ethanol:water 4:1:5 mixture being employed as solvent. In some preliminary experiments, good separations of β-D-galactoside, D-galactose, ~~methyl~~ 6-O-methyl-D-galactose and 1,6-anhydro-β-D-galactose from one another were obtained, the spots being shown up using $AgNO_3$/NaOH reagent[74].* On examination of a concentrate from the aqueous solvolysis of the β-galactoside tosylate however, extensive streaking was encountered, an intense spot of $R_F$ 0.37, corresponding to none of the above standards, standing out from the streaky background. Neutralisation of the reaction products with an anion exchange resin Amberlite IR-45 prior to concentration showed that the streaking was not an artefact due to the presence of the toluene-p-sulphonic acid, but was caused by at least one of the carbohydrate end products.

In view of the acid-labile nature of the glycoside link in 3,6-anhydro compounds[75] the possible presence of 3,6-anhydro-D-galactose was investigated. Since during the solvolysis, the solution components were subjected to an increasingly acidic environment, a hydrolysis of this kind seemed not unlikely. In fact, it was found that the syrupy reaction products were strongly reducing, Tollen's Reagent (ammoniacal silver nitrate) producing a silver mirror.

* Note: Methyl 3,6-anhydro-β-D-galactose did not produce a spot with $AgNO_3$/NaOH. No suitable reagent was found during this period.

In a control experiment methyl 3,6-anhydro-β-D-galactoside in M/300 toluene-p-sulphonic acid was kept at 50°C for 5 days, the acid removed with anionic-exchange resin, and the solution concentrated in the same manner as the solvolysis products. Chromatography by the above technique ($AgNO_3$/NaOH reagent) gave exactly similar streaking, with a concentrated spot $R_F$=0.37. In a final chromatographic confirmation, a sample of the syrupy 3,6-anhydro-D-galactose was prepared (identified as its diethyl mercaptal[76]), and a chromatogram run against the solvolysis products. Again, a similar effect was obtained, an additional spot $R_F$= 0.14 arising, presumably, from the formation of reversion products of 3,6-anhydro-D-galactose during hydrolysis of the glycoside.

Following some observations of Araki[75] on the behaviour of 3,6-anhydro-aldehydo-D-galactose in dilute sulphuric acid, a sample of the distillate from the solvolysis products was examined spectrophotometrically in the ultraviolet. No peak at 285mμ was observed, indicating the absence of measurable amounts of hydroxymethyl furfural in the solution. More vigorous conditions are obviously required for the dehydration-rearrangement reaction to occur.

Chemical Analysis. The presence of a 3,6-anhydro-D-galactose derivative in the solvolysis products was confirmed by reaction of the syrupy concentrate with ethyl mercaptan in concentrated hydrochloric acid. Crystalline 3,6-anhydro-D-galactose diethyl mercaptal was isolated from the reaction products[76]. It was thus shown that the primary reaction of methyl 6-O-tosyl-β-D-galactoside in water at 50° is an intramolecular $S_N2$ displacement of tosylate by the 3-hydroxyl group. Owing to the extremely long reaction times, the end products of the solvolysis of the other three tosylates were not examined, and it was assumed, from the point of view of interpreting the kinetics, that the primary reaction, namely the release of $OTs^-$ ion, involved the same mechanism in all cases. Bearing in mind that no indication of

products other than 3,6-anhydro derivatives arose from the above studies, the chances of a complete changeover in mechanism on altering configuration seems remote.

Kinetic Procedure

The kinetic experiments, which, as mentioned above, were carried out during preliminary investigations, are not claimed to be precise; the sources of error were many. Some of the differences in reactivity observed were, however, well outside experimental error, and the semi-quantitative results on which the discussion is based are certainly valid.

Runs at room temperature. Following the method of Baker for 3M salt solutions, methyl 6-O-tosyl-α- and -β-D-galactosides were allowed to react in water at room temperature, the release of tosylate being followed spectrophotometrically. Readings were taken about once a week over a nine week period, this period corresponding to about two half-lives for the β-anomer but only about half of a half-life for the α-compound. The glucoside 6-tosylates both reacted at an impossibly slow rate at this temperature.

Using values of $E_\infty$ (see equation on p. 45 ) calculated on the assumption that only tosylate ion was being measured, surprisingly good first-order kinetics were obtained for the two galactosides over the period examined, approximate rate constants showing a quite definite differential in the reactivities of the anomers.

Runs at 50°. Hopes that this elevated temperature would increase the relative solvolysis rates sufficiently to permit accurate kinetic studies were not, unfortunately, realised. While the rate of the β-galactoside reaction (half-life ca 16 hours) could be measured with some accuracy, the glucosides were still hopelessly unreactive, readings over only fractions of a half-life being obtained during ten days.

The 50° reactions were followed conductometrically, the drop in resistance of

aqueous solutions of the tosylates being measured across platinised platinum electrodes, by means of a conductivity bridge. The results were plotted according to the 1st order equation calculated for conductivity measurements:

$$k = \frac{2.303}{t} \log_{10} \frac{\lambda_\infty - \lambda_0}{\lambda_\infty - \lambda_t}$$

where $\lambda_\infty$, $\lambda_0$ and $\lambda t$ are conductivies at times $\infty$ , 0 and t respectively

Approximate linearity of t against $\lambda_\infty - \lambda t$ was obtained over 4 and 2 half-lives for methyl 6-O-tosyl-β-D-galactoside and methyl 6-O-tosyl-α-D-galactoside respectively using end-values ( $\lambda_\infty$ ) obtained experimentally.

Since quantitative evaluation of the end products was not possible a check on the nature of the conducting species produced during the solvolysis of the galactosides was made by calculation of the end-values from a plot of conductivity against concentration for solutions of toluene-p-sulphonic acid. Assuming one mole of toluene-p-sulphonic acid was released by one mole of tosylate, the end-values of the β-galactoside solvolysis from experiment and calculation were in good agreement. In the case of the α-galactoside, however, the theoretical end-value was reached after only ca 2 half-lives, the conductivity continuing to rise to a value in excess of that predicted by calculation. There appeared to be a slow secondary reaction involving the production of conducting species (possibly 3,6-anhydro-D-galactonic acid) which, unfortunately, interfered with the rate-measurements of the primary reaction. The rate constant will, however, lie between the figures calculated using the experimental and theoretical end-values.

In view of the uncertainties brought about by this interfering side reaction, rate constants for the glucoside 6-tosylates calculated from data within the first half-life and using a theoretical end-value, are quoted only to demonstrate the low reactivity of these compounds as compared with the galactosides. They are of little significance in any quantitative sense.

Results and Discussion

The approximate first-order rate constants for the aqueous solvolysis of methyl 6-O-tosyl-β- and -α-D-galactosides and methyl 6-O-tosyl-β- and -α-D-glucosides at 50° are given in Table 5 , and the rate constants for the galactosides at room temperature in Table 6 .

Two general conclusions can be drawn from these rate figures. The galactosides are many times more reactive in their aqueous solvolysis than the glucosides, and the β-galactoside is several times more reactive than the α-anomer. On conformational grounds, the first observation is reasonable, and in agreement with the analogous results in alkali, The axial-equatorial shift of the 4-hydroxyl which occurs for galactosides during the requisite C1 — 1C conformational change produces an accelerating influence absent for the glucosides (c.f. p. 16 ). The inequality β-galactoside > α-galactoside for rates of solvolysis seems, at first sight, a contradiction, since the axial to equatorial change of the α-methoxyl might be expected to accelerate the reaction of this anomer by a similar steric mechanism. (c.f. the reactions in alkali, in which, for glucoside and galactoside 6-tosylates, the α-anomers react ca 1.5 times faster than the corresponding β-anomers). This reversal in order may be interpreted as follows. As discussed in Part I of this work, conformational stability in aqueous systems is determined mainly by steric and dipole influences. Thus, when a galactoside changes from its stable C1 conformation to the reactive 1C conformation, these two influences tend to act in opposition on the anomeric methoxyl. For α-galactosides, steric effects tend to encourage the interchange, while the dipole effect exerts a stabilising action on the axial anomeric methoxyl; for β-galactosides, the reverse is true. It is proposed here that for the cyclisation of galactoside 6-tosylates in alkali, the stability of the 1C conformation is determined predominantly by the bulky hydrated 3-oxy anion, steric

TABLE 5

The rates of solvolysis of methyl 6-O-tosyl-α- and -β-D-galactoside and of methyl 6-O-tosyl-α- and -β-D-glucoside in water at 49.3 ± 0.2°

| Compound | Conc'n of Compound | $10^6 k$ ($sec^{-1}$) |
|---|---|---|
| Methyl 6-O-tosyl-α-D-galactoside | 0.001M | 2.2 (exptl) * |
| " | " | 3.6 (calc.) 7 |
| Methyl 6-O-tosyl-β-D-galactoside | 0.005M | 12 (both ) |
| Methyl 6-O-tosyl-α-D-glucoside | " | 0.4 (calc.) |
| Methyl 6-O-tosyl-β-D-glucoside | " | 0.5 (calc.) |

TABLE 6

The rates of solvolysis of methyl 6-O-tosyl-α and -β-D-galactoside in water at room temperature (about 21°)

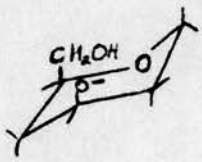
| Compound | Conc'n of Compound | $10^3 k$ ($hours^{-1}$) |
|---|---|---|
| Methyl 6-O-tosyl-α-D-galactoside | $0.25 \times 10^{-3}$M | 0.2 (calc.) |
| Methyl 6-O-tosyl-β-D-galactoside | " | 1.0 (calc.) |

* using experimental end-value

7 using calculated end-value

influences overriding the dipole effect, and causing a net increase in the relative reactivity of the α-anomer.

"Reactive" conformations




In alkaline solution


In neutral or acidic solution

For the neutral reaction, the steric "bulk" of the smaller 3-hydroxyl group would then be insufficient to overcome the dipole effect, causing a relative increase in the stability of the β-anomer in the $^1C$ conformation, and consequently a higher reactivity of this anomer as compared with the α-compound.

According to the above considerations, the β-glucoside 6-tosylate should solvolyse much faster than the α-anomer, but from the approximate rates obtained, they appear to be of similar reactivities. However, considering the large errors introduced owing to the slowness of the reactions, and the probable contributions of secondary reactions, the rate differences may be obscured. It is also possible that for the extremely slow reactions, other reactions (e.g. $S_N2$ attack by water) may contribute appreciably to the primary process. The effect of the increasingly acidic conditions on the rates of cyclisation is not known. In view of the observation by Brown and Timmis[77] that certain polyol sulphonates cyclise to anhydro-derivatives under acidic conditions, the possibility that progressive acid catalysis occurs during the solvolysis must also be taken into account.

It would obviously be of great interest to measure the solvolysis rates of a wide range of 6-tosylates for comparison with the reactions in alkali, but the

difficulties are seen to be manifold. The main trouble, as has been noted, is due to the slowness of some of the reactions, extended reaction times tending to encourage secondary reactions, thus confusing kinetic measurements and product analysis. While this difficulty could be reduced by the use of more reactive sulphonates, p-nitro-benzene sulphonates for example, such systems would not be strictly comparable to the tosylates and a repetition of all the kinetic work in alkali using p-nitro-benzene sulphonates would hardly seem worthwhile. Further, compounds having the desired degree of reactivity would tend to be unstable, and therefore difficult to isolate.

The project was therefore discontinued at this stage.

## EXPERIMENTAL

### Preparative Work

The 6-tosylates used in the above investigation, and for the preparation of methyl 3,6-anhydro-α- and -β-D-galactosides, were kindly supplied by R.Baker.

### Methyl 3,6-anhydro-β-D-galactoside [78]

Methyl 6-O-tosyl-β-D-galactoside (1.21g.) was ground to a powder, dissolved in ethanol (7.5 ml.) and the solution treated with N aqueous sodium hydroxide (4 ml.). The mixture was stood overnight at room temperature, and then warmed to 60° for 1½ hours to complete the reaction. The solution was then neutralised with solid carbon dioxide, and evaporated to dryness under reduced pressure. The resulting white solid was extracted with portions of warm acetone (3 x 20 ml.), the combined extracts filtered, and the filtrate evaporated to dryness under reduced pressure to give the crude product in crystalline form. Recrystallisation from acid-free ethyl acetate gave methyl 3,6-anhydro-β-D-galactoside (0.35g.) in white prisms m.p. 117.5 - 118° (Lit: m.p. 118°). [78]

Methyl 3,6-anhydro-α-D-galactoside [78]

Methyl 6-O-tosyl-α-D-galactoside (4.0g.) was treated as described above for the β-anomer, the reaction mixture being shaken mechanically because of the relative insolubility of the starting material. The remaining solid dissolved on warming, and after working up in the usual way, and crystallising from ethyl acetate, methyl 3,6-anhydro-α-D-galactoside (1.1g.) was obtained in fine white needles m.p. 140° (Lit: m.p.140°).[78]

3,6-anhydro-D-galactose diethyl mercaptal [76]

Methyl 3,6-anhydro-α-D-galactoside (100 mg.) was dissolved in concentrated hydrochloric acid (0.15 ml.) precooled to 0°. Ethyl mercaptan (0.1 ml.) was added, and the mixture was shaken at 0° for 1½ hours. The solution was then diluted with ice-water, and the precipitated solid filtered off and washed with cold water. The crude material was dried *in vacuo* over solid potassium hydroxide, and crystallised from ethyl acetate to give 3,6-anhydro-D-galactose diethyl mercaptal (100 mg.) in white needles m.p. 111 - 112° (Lit: m.p. 112 - 113°).[76]

From the solvolysis products

Methyl 6-O-tosyl-β-D-galactoside (200 mg.) was solvolysed in distilled water (150 ml.) for 5 days at 50°. The solution was neutralised with anionic-exchange resin, and the water removed at 40° under reduced pressure. The syrupy concentrate was treated with concentrated hydrochloric acid and ethyl mercaptan as described above. On dilution, only a very small precipitate was formed, so the aqueous mixture was extracted with several 20 ml. portions of chloroform, the extracts combined, and dried over anhydrous sodium sulphate. Removal of the chloroform gave a white solid (*ca.* 20 mg.) which on crystallisation from ethyl acetate gave 3,6-anhydro-D-galactose diethyl mercaptal (12 mg.) m.p. 112 - 113°, mixed m.p. with sample prepared above 112 - 113°.

3,6-anhydro-D-galactose[75]

Methyl 3,6-anhydro-α-D-galactoside (0.4g.) was dissolved in 0.1N sulphuric acid (5 ml.), and the solution stood at room temperature for 24 hours. Excess barium carbonate was then added to neutralise the acid, inorganic material was filtered off, and the filtrate evaporated to dryness under reduced pressure. Extraction with acetone followed by evaporation of the extract gave a syrup (0.3g.) which reduced Tollens reagent (ammoniacal silver nitrate) to a silver mirror, and which reacted with concentrated hydrochloric acid/ethyl mercaptan according to the above method to yield the diethyl mercaptal. The syrup thus consisted mainly of 3,6-anhydro-aldehydo-D-galactose.

Paper Chromatography

Paper chromatography was carried out by the descending technique on strips of Whatman No.1 paper, the solvent system being the top layer of a butanol:ethanol:water 4:1:5 mixture. Chromatograms run for 20-24 hours at room temperature were found to give reproducible $R_f$'s for the compounds studied. Spots were shown up by dipping the dried strips in a silver nitrate/acetone solution (0.1 ml. saturated aqueous silver nitrate in 20 ml. acetone), drying the papers in the air, and spraying uniformly with a 2 % solution of sodium hydroxide in aqueous ethanol ( 2 g. solid NaOH in 2 ml. water, diluted with 98 ml. absolute ethanol). In most cases, brown spots on a white background were obtained.

The following $R_f$ values were obtained for the compounds studied:-
D-galactose: 0.11 - 0.13. Methyl-β-D-galactoside: 0.24 - 0.25
6-O-methyl-D-galactose: 0.27. 1,6-anhydro-β-D-galactose: 0.41.
3,6-anhydro-aldehydo-D-galactose (intense spot): 0.37.

Kinetic Experiments

At room temperature

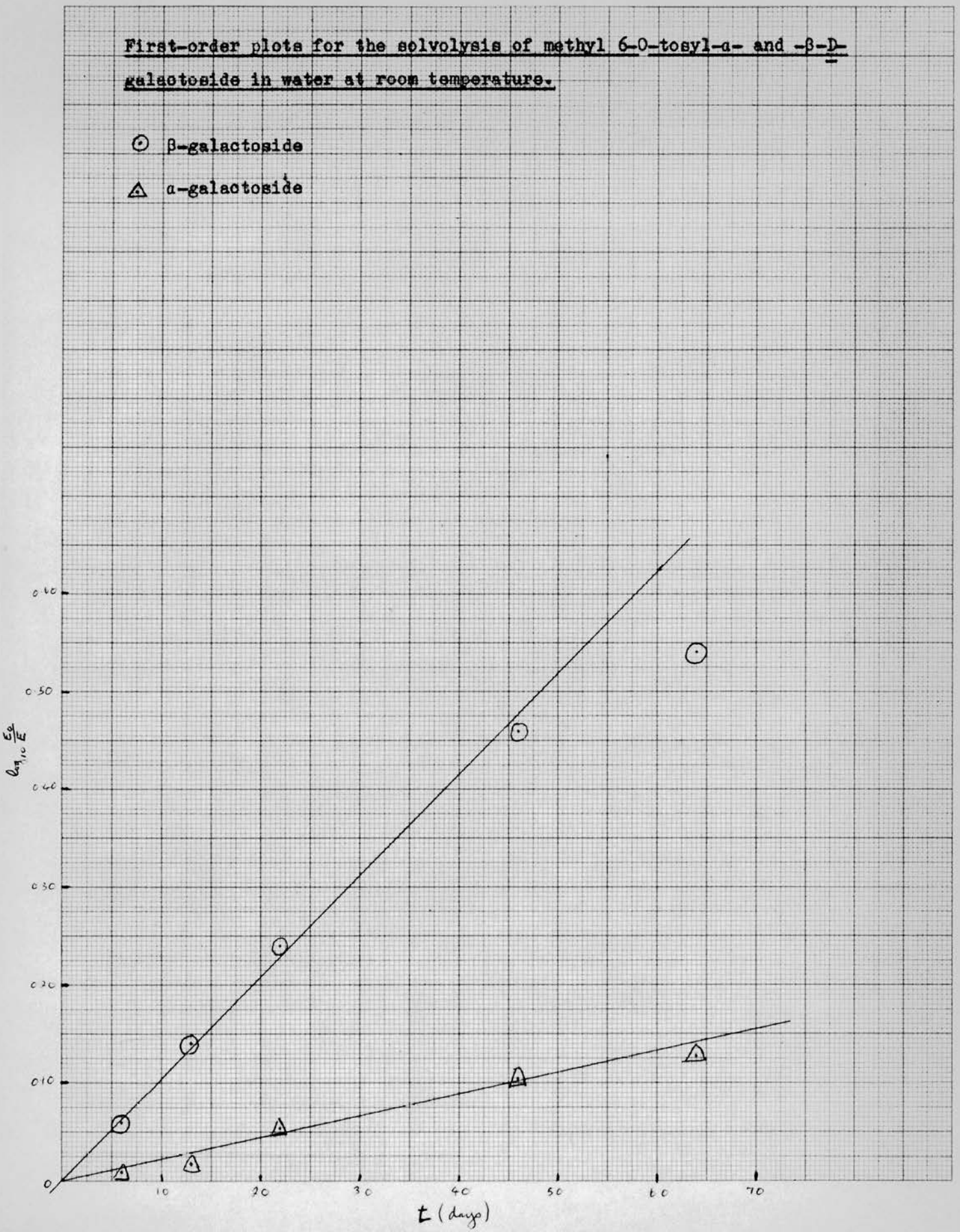
The conversion of tosylate ester to tosylate anion was followed spectrophotometrically at 265mμ as described for the secondary tosylates in Part I (p.45 ); as described therein, a sodium tosylate blank was used. Readings were taken at approximately 7 day intervals, and for the α- and β-D-galactosides, the data were processed according to the usual first-order equation (p. 45 ), an end-value of zero being assumed. Reasonably acceptable linearity was obtained over the period examined, graphs for the two galactosides being shown on page 92. Pure chemicals, deionised water and scrupulously clean apparatus were used to minimise slow reactions with contaminants, and the cells were securely stoppered to eliminate air and bacteria as far as possible.

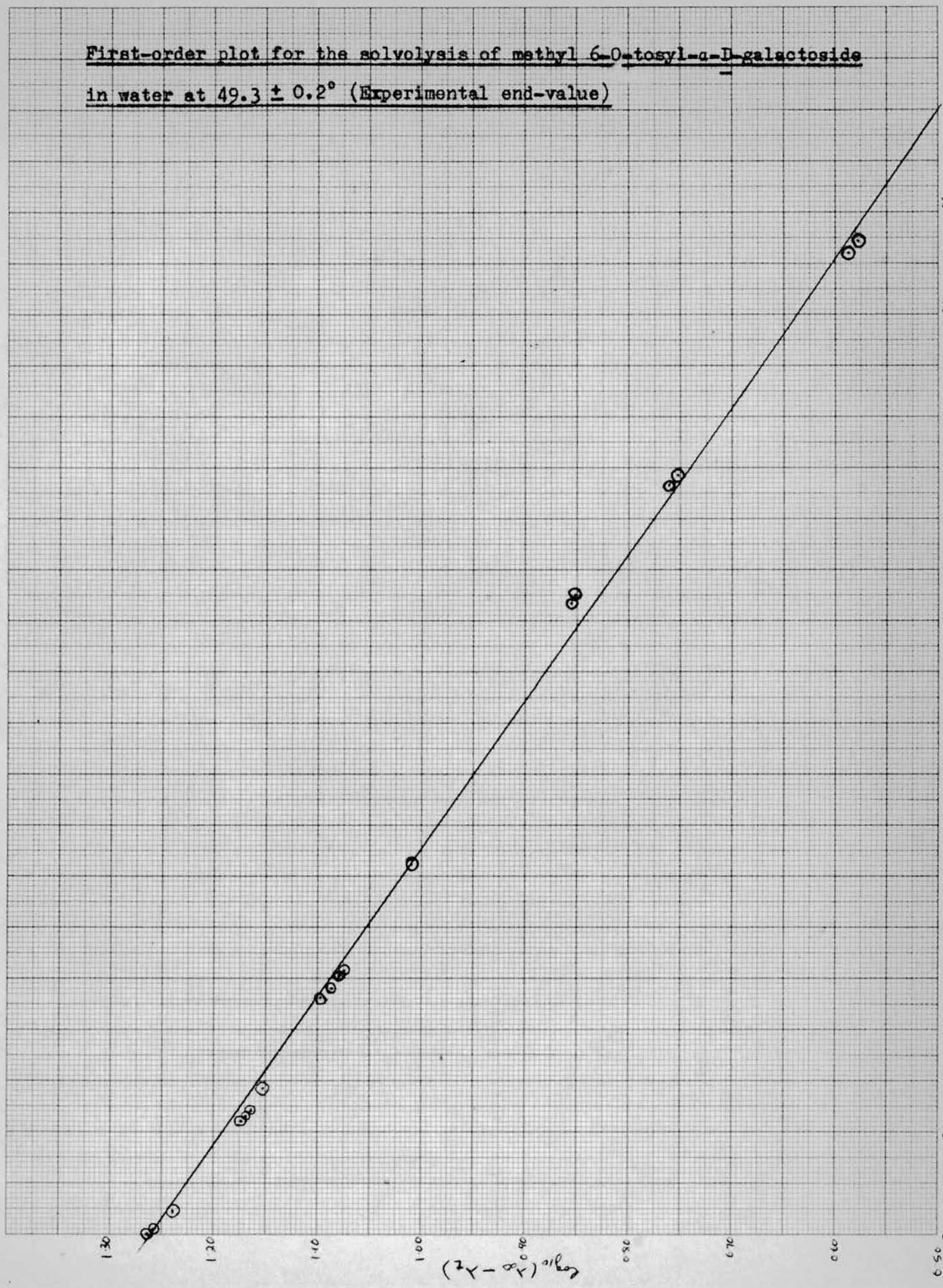
At 50° Changes in conductance of 0.005M solutions of the tosylates in carbonate-free distilled water were measured at temperatures in the range 49.10 - 49.50°, thermostatting being provided by a water-bath/Tempunit system. The conductivity cell employed was a simple one-compartment unit with platinised platinum electrodes one centimetre square and about one centimetre apart. Changes in conductivity were measured using a Tinsley Type 4896 Conductometric Bridge reading to $10^{-1}\,\Omega$, regular A.C. current being provided by a bridge oscillator. The null point was detected by means of earphones.

For each run, readings were taken at regular intervals during each day over a 6 day period, and results were plotted according to the appropriate first-order equation (p.83 ). Fairly good linearity was obtained (see, for example, page 93. ) over the periods examined, although for the glucosides, the periods amounted to fractions of one half-life. End-values were obtained by experiment for the α-and β-D-galactoside 6-tosylates, and compared with those calculated on the

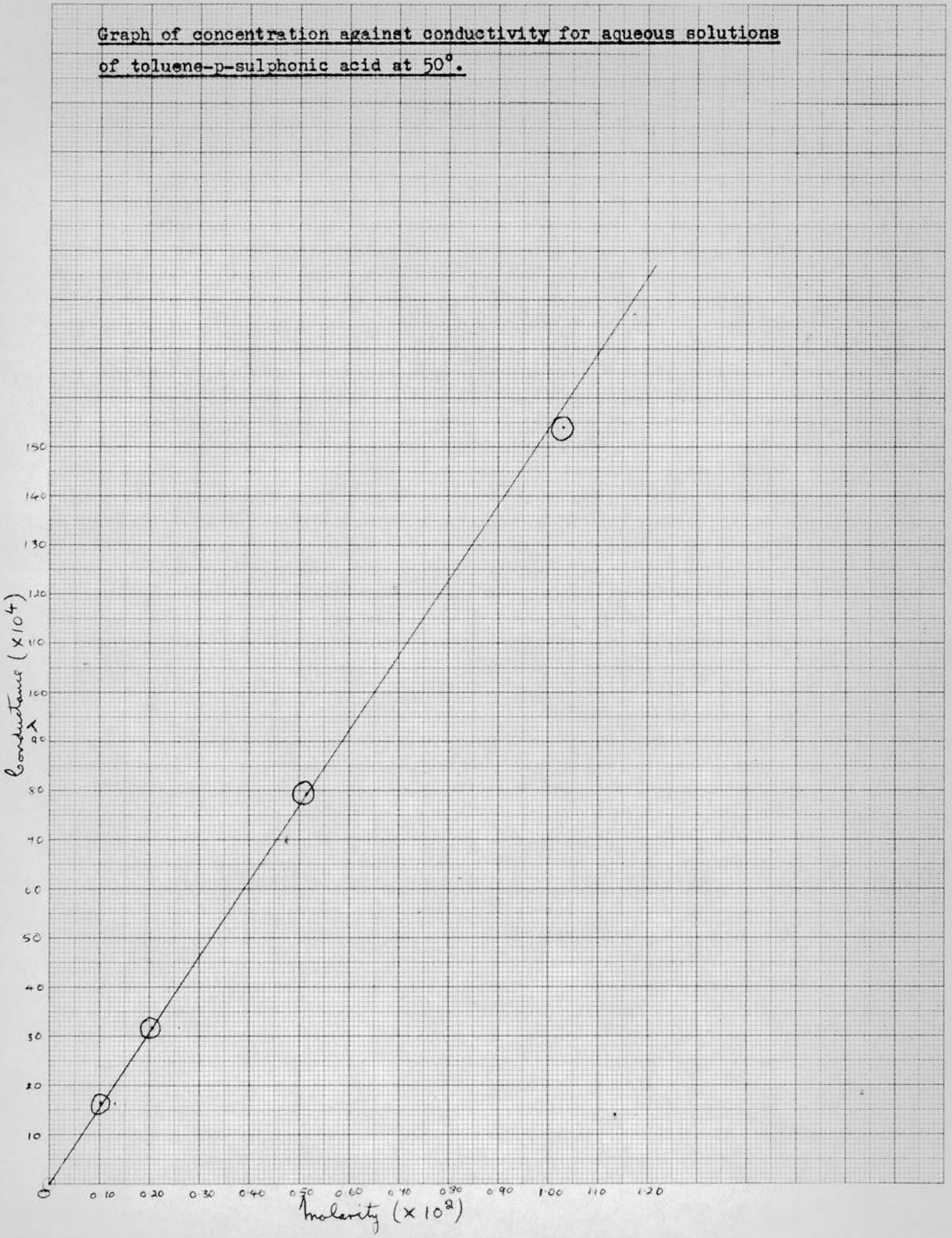
basis of a calibration graph of conductivity/concentration of toluene-p-sulphonic acid (p. 93a). Due to the extremely low reactivites of the glucoside 6-tosylates, approximate rate constants for the solvolyses of these compounds were evaluated using calculated end-values.

First-order plots for the solvolysis of methyl 6-O-tosyl-α- and -β-D-galactoside in water at room temperature.




First-order plot for the solvolysis of methyl 6-O-tosyl-α-D-galactoside in water at 49.3 ± 0.2° (Experimental end-value)
log10 (λ∞ − λt)
t (hours)

Graph of concentration against conductivity for aqueous solutions of toluene-p-sulphonic acid at 50°.



## PART III

### Some Experiments on the Alkaline Fragmentation of Methyl 2-O-tosyl-α-D-glucoside

As mentioned in the Introduction, the cyclisation of methyl 2-O-tosyl-α-D-glucoside in aqueous sodium hydroxide does not proceed smoothly, appreciable amounts of material absorbing strongly in the U.V. being formed. Although not strictly within the province of this work, some experiments were carried out in an attempt to identify the products of this secondary reaction, and hence to elucidate the reaction mechanism. The analysis was approached in several ways, and in the following section, experiments are outlined under the headings of the techniques employed.

#### Ultraviolet Spectroscopy

The rise in absorption at 265mμ of a $5 \times 10^{-5}$M solution of methyl 2-O-tosyl-α-D-glucoside in 0.5N sodium hydroxide at 25° followed approximately first-order kinetics, a rate constant of about $2.8 \times 10^{-4}$ sec.$^{-1}$ being obtained. The U.V. spectrum of the reacted solution in a 1 cm. cell, corrected for the presence of tosylate ion, showed an absorption maximum at 267-268mμ of about 0.6 optical density units. On acidification with sulphuric acid, a hypsochromic wavelength shift occurred, the maximum moving to 246-247mμ, with an equivalent optical density (corrected for dilution) of about 0.2 units. The optical density of the maximum in weak sulphuric acid (about 0.01N) was found to rise steadily, increases of about 5, 25, 50 and 80% of the original optical density being observed at times of 20 minutes, 2 hours, 24 hours and 4 days from the time of acidification. Rebasification of the solutions after the above times, as well as immediately after

acidification, showed that the original products were not restored. The absorption at the maximum of the rebasified initial solution was only some 55% of the original value, the optical densities in alkali of the acid-treated solutions rising in parallel with those of the maxima in acid. The spectra of all the alkaline solutions, including the original one in 0.5N-NaOH, were relatively stable.

From these results, it appeared that the system underwent two reactions in acid; an initial extremely rapid reaction causing the depression and shift of the maximum in alkali, followed by a slow reaction producing material absorbing in a similar manner to the original reaction mixture.

Polarimetry

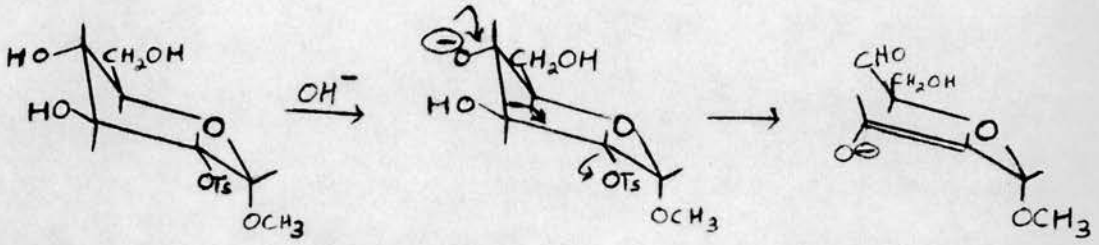
The optical rotation of a 0.029M solution of the tosylate in 0.5N-NaOH measured at 21° fell from +1.59 to a constant value of +0.93. The rotation change followed reasonable first-order kinetics, a rate constant of the same order as that obtained spectroscopically being derived from the data. On acidification, a further drop in rotation was observed, a value of+0.31 being noted after 24 hours. It was therefore considered that the changes in U.V. absorption of the acidified reaction products were probably due, at least in part, to chemical modification of some optically active species.

The presence of methyl 2,3-anhydro-α-D-mannoside in the products was demonstrated by paper chromatography using 4:1:5 butanol:ethanol:water. A solution of this compound showed very weak absorption in the ultraviolet region, and the stability of its spectrum in acidic and alkaline environments indicated that the epoxide was not an intermediate in the formation of the highly-absorbing materials.

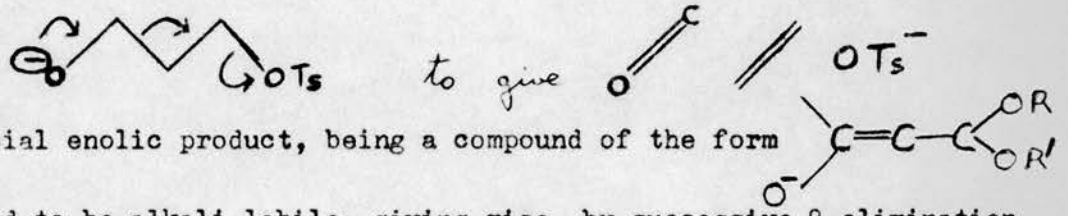
With the above information on hand, a possible mechanism was sought with a view to devising suitable techniques for chemical analysis. In the light of the above evidence, and much experimental information available on the reactions of sugar epoxides

in alkali, it was considered that the highly-absorbing species were unlikely to be formed *via* the epoxide, and that a different primary process was occurring in competition with epoxide formation. Since protection by means of a 4,6-O-acetal link has been shown to allow quantitative conversion to the 2,3-epoxide, a mechanism involving the ionisation of the 4-hydroxyl was proposed, closely analogous to a fragmentation reaction of fairly common occurrence.[80] As will be seen, this reaction could give rise to enolic materials with U.V. absorption characteristics in acid and alkali similar to those obtained for the reaction products.

The primary stage in a fragmentation of this type, as applied to methyl 2-O-tosyl-α-D-glucoside, may be represented as follows:




the essential reaction involving the formation of a 5-centre coplanar transition state




This initial enolic product, being a compound of the form



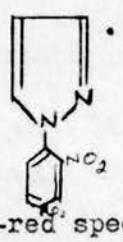
would be expected to be alkali-labile, giving rise, by successive β-elimination and hydrolysis, to D-glyceraldehyde, malondialdehyde (HO - CH = CH - CHO in its enolic form), and methanol. While many other reactions in acid or alkali, involving rearrangements, condensations, eliminations, Cannizzaro reaction, etc., may be proposed, it was hoped that analysable amounts of D-glyceraldehyde and malondialdehyde would be present in the reaction products. The U.V. spectra of malondialdehyde in acid and alkali were therefore measured, and some chemical methods for the analysis of the two aldehydes investigated.

The U.V. spectra of malondialdehyde

Malondialdehyde was prepared by the hydrolysis of the diacetal, 1,1,3-trimethoxy-3-ethoxy-propane, in 3% aqueous sulphuric acid.[81] The ultraviolet spectra in acid and alkali displayed maxima at 245mμ (ε 13,500) and 266mμ (ε 26,400) respectively. Although there were differences in the relative absorption intensities in acid and alkali between malondialdehyde and the fragmentation products, the main features of the spectra were very similar, and it was estimated that 30 - 50% reaction to the enol would account for the spectra of the products. This was in good agreement with the value (30%) calculated from the final rotation of the products in alkali, assuming D-glyceraldehyde and methyl 2,3-anhydro-α-D-mannoside, to be the only optically active products.

Chemical Analysis

Malondialdehyde in the free state has been shown to react with phloroglucinol/concentrated HCl,[82] and with 2-thiobarbituric acid and some derivatives thereof,[83] to give coloured products. However, the colours produced by these reagents lack the specificity required for unambiguous identification, and an attempt to isolate crystalline derivatives was made.

As reported in the patent literature, the malondialdehyde diacetal was found to react rapidly with aniline/dilute hydrochloric acid to give the orange crystalline dianil hydrochloride which, on treatment with sodium hydroxide, yielded the yellow-orange crystalline dianil (Ph-NH-CH=CH-CH=NPh).[84] On treatment with Brady's reagent (2,4-dinitrophenylhydrazine in aqueous hydrochloric acid) at less than 5°, the acetal reacted to give a crystalline red-brown solid, presumed, from its physical properties, to be 2,4-dinitrophenylpyrazole,* 

.[85] Under similar conditions, this

* A small NH peak in its infra-red spectrum indicated that small amounts of some contaminant were present.

reagent combined with glyceraldehyde to give glyceraldehyde dinitrophenylhydrazone.[86] Since the latter two compounds were found to be separated efficiently by chromatography on a column of activated silica gel, this approach seemed particularly promising.

Application of the above techniques to the 2-tosylate/NaOH reaction products was, however, found to give disappointing results. Aniline/HCl reacted very slowly to give a dark brown amorphous solid which resisted all attempts at purification. Brady's reagent, on the other hand, gave an immediate brick red precipitate which, on microscopic examination, was seen to consist of at least two components, the major one being bright red, but some specks of yellow-orange materials being present. It was subsequently found that free malondialdehyde behaves differently from the diacetal towards these reagents, the products tending to be inhomogeneous and non-crystalline.

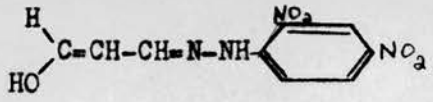
Column Chromatography

The crystalline mixture obtained on reaction of the fragmentation product with Brady's reagent was subjected to chromatography on columns of activated silica gel, and of neutralised alumina. No useful separation of the mixture was obtained on silica gel, but on activated alumina, resolution into four components was obtained. Only one of these was obtained in sufficiently large amounts for further examination. Reaction of the hydrolysed diacetal of malondialdehyde with Brady's reagent gave a solid mixture which, on alumina chromatography, gave a small amount of an unidentifiable yellow solid, indicating that the brick-red solid was not derived from free malondialdehyde.

The ultraviolet spectrum of the unknown dinitrophenylhydrazone

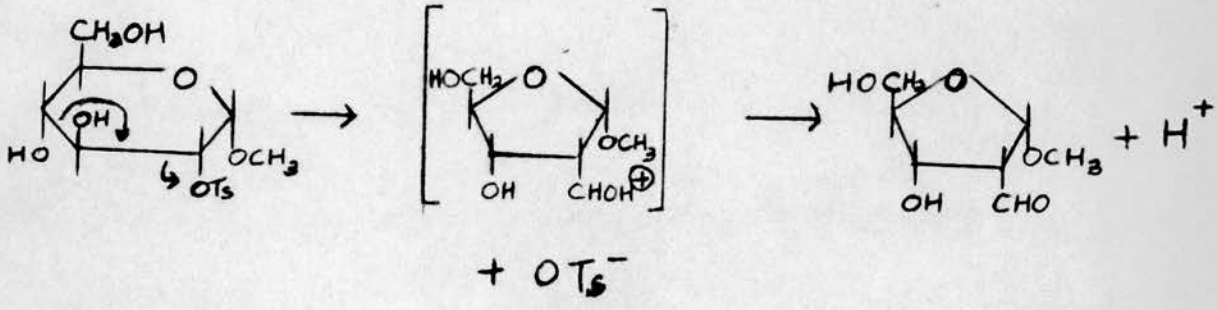
The U.V. and visible spectrum of the brick red solid eluted from the alumina column as described above was examined in neutral and alkaline solution (see p.105). Interpreted according to Timmons,[87] the positions and relative extinctions of the

$E_0$, $E_1$ and $E_2$ bands in neutral solution, and of the $E_0^1$, $E_1^1$ and $E_2^1$ bands in alkali indicated that the compound is a 2,4-dinitrophenylhydrazone of a biconjugated unsaturated aldehyde. The suggestion that this compound may be

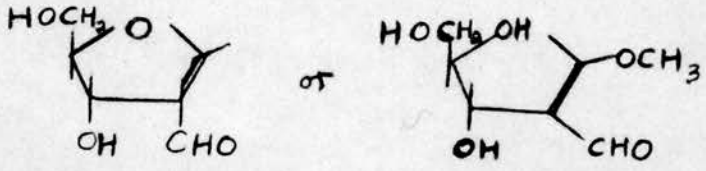


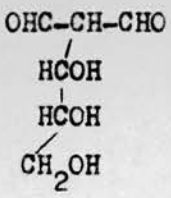
is pure supposition, and lack of time precluded further study of this material. However, chemical analysis added some support to this postulated structure.

The failure to isolate any malondialdehyde or glyceraldehyde derivatives from the reaction mixture cast some doubts on the originally proposed mechanism. In addition, the spectroscopic and polarimetric behaviour of the products in acid solution does not fall in line with the expected reactions. A more detailed study is required, and further reagents, selective where possible, need be sought. All possible mechanisms will have to be examined in detail. By analogy with a reaction recently encountered by Buchanan[88] in the solvolysis of some 3-O-p-nitrobenzenesulphonyl glycosides, one such mechanism is proposed. It would involve displacement of the tosyloxy group by ring contraction, the attacking group being carbon-4, i.e.



This aldehyde could then undergoβ-elimination at carbon-1 or carbon-3, the possible products from the first of these being



Either of these, on hydrolysis would yield the optically active malondialdehyde derivative  . An examination of the products with 2,4-dinitrophenylhydrazine for optical activity would clearly be of interest. It is not, however, clear how the above reaction would be catalysed by alkali; it could be postulated that ionisation of the 4-hydroxyl group increases the nucleophilicity of carbon-4, but there appears to be no precedent for this.

## EXPERIMENTAL

### Preparative Work

#### Methyl 2-O-tosyl-α-D-glucoside [89]

Methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucoside (500 mg.) prepared as described on p. 30 was dissolved in glacial acetic acid (6 ml.), the solution heated on a steam-bath, and distilled water (4 ml.) added dropwise. After heating for one hour, distilled water (10 ml.) was added to the mixture, and the solution evaporated to dryness under reduced pressure at 60°, last traces of water being removed by codistillation with dry benzene. The residual syrup crystallised on rubbing with a glass rod, and crystallisation from ethyl acetate/petroleum ether gave methyl 2-O-tosyl-α-D-glucoside (320 mg.) in white prisms m.p. 137 - 139° (Lit: m.p. 139 - 140°).

#### Malondialdehyde dianil [84]

1,1,3-trimethoxy-3-ethoxy propane (0.4g.) in 2N-hydrochloric acid (40 ml.) was treated with pure aniline (0.6g.) for 24 hours at room temperature. The orange precipitate was filtered off, washed with cold distilled water, and dried to give malondialdehyde dianil hydrochloride (0.4g.) m.p. 210° (Lit: m.p's between 209 and 216°).[84] The hydrochloride was shaken with a mixture of ether and excess dilute sodium hydroxide solution for five minuts, the ether layer separated, washed with water, and dried over

anhydrous sodium sulphate, and the clear yellow solution evaporated to low volume. Addition of petroleum ether precipitated malondialdehyde dianil (0.3g.) m.p. 114 - 115° (Lit: m.p's between 112 and 115°).[84]

2,4-dinitrophenyl pyrazole [85]

1,1,3-trimethoxy-3-ethoxy propane (0.12g.) was dissolved in water (20 ml.), the solution cooled to below 5°, and a similarly precooled solution of Brady's Reagent (0.5% 2,4-dinitrophenylhydrazine in 2N aqueous hydrochloric acid) (50 ml.) was added. The solution darkened almost at once, and within 2 minutes, a copious red-brown precipitate had formed. After several hours, the solid was filtered off, washed with distilled water, dried and crystallised from ethyl acetate/petroleum ether to give reddish crystals (100 mg.) m.p. 113 - 115°. This was taken to be pure 2,4-dinitrophenyl pyrazole (Lit: m.p. 107 - 109°),[85] although a small NH peak in the infrared spectrum of the solid indicated some inhomogeneity.

Reaction of free malondialdehyde with the above reagents.

1,1,3 trimethoxy-3-ethoxy propane (0.1g) was hydrolysed with N-hydrochloric acid (50 ml.) for four hours at room temperature, and the solution neutralised with N-sodium hydroxide solution. Portions of the neutral solution were treated as described above with aniline/hydrochloric acid, and with Brady's reagent. Products from both reagents were found to be inhomogeneous, and not purifiable by a few recrystallisations from ethyl acetate/petroleum ether.

Reaction of fragmentation products with the above reagents.

Methyl 2-O-tosyl-α-D-glucoside (1.0g.) was reacted for one day at room temperature with 0.5N-sodium hydroxide (20 ml.), and the reaction mixture neutralised with dilute hydrochloric acid.

Aniline/hydrochloric acid. One half of the above solution was treated with 5N hydrochloric acid (7.5 ml.) and aniline (0.5 ml.). A small amount of amorphous dark-brown solid separated from the yellow solution after 48 hours reaction at room

temperature. Attempts at purification by crystallisation proved unsuccessful.

Brady's reagent. The remaining half of the neutralised reaction solution was treated with Brady's reagent (50 ml.) at below 5°, as described above. A copious brick-red precipitate was formed almost at once, microscopic examination of the separated solid showing that at least two components (red and yellowish in colour) were present. Attempted resolution of the mixture by fractional recrystallisation met with little success, but at least partial separation was achieved by adsorption chromatography on alumina, as described shortly.

Glyceraldehyde 2,4-dinitrophenylhydrazone [86]

Glyceraldehyde (0.1g.) was dissolved in distilled water (25 ml.), the solution cooled to below 5°, and treated with a precooled solution of Brady's reagent (60 ml.). After reaction overnight at 5°, the yellow solid was filtered off, washed with a little water, and crystallised from ethyl acetate/ethanol/water to give glyceraldehyde 2,4-dinitrophenylhydrazone (0.26g.) m.p. 164 - 166° [86] (Lit: m.p. 164 - 166°).

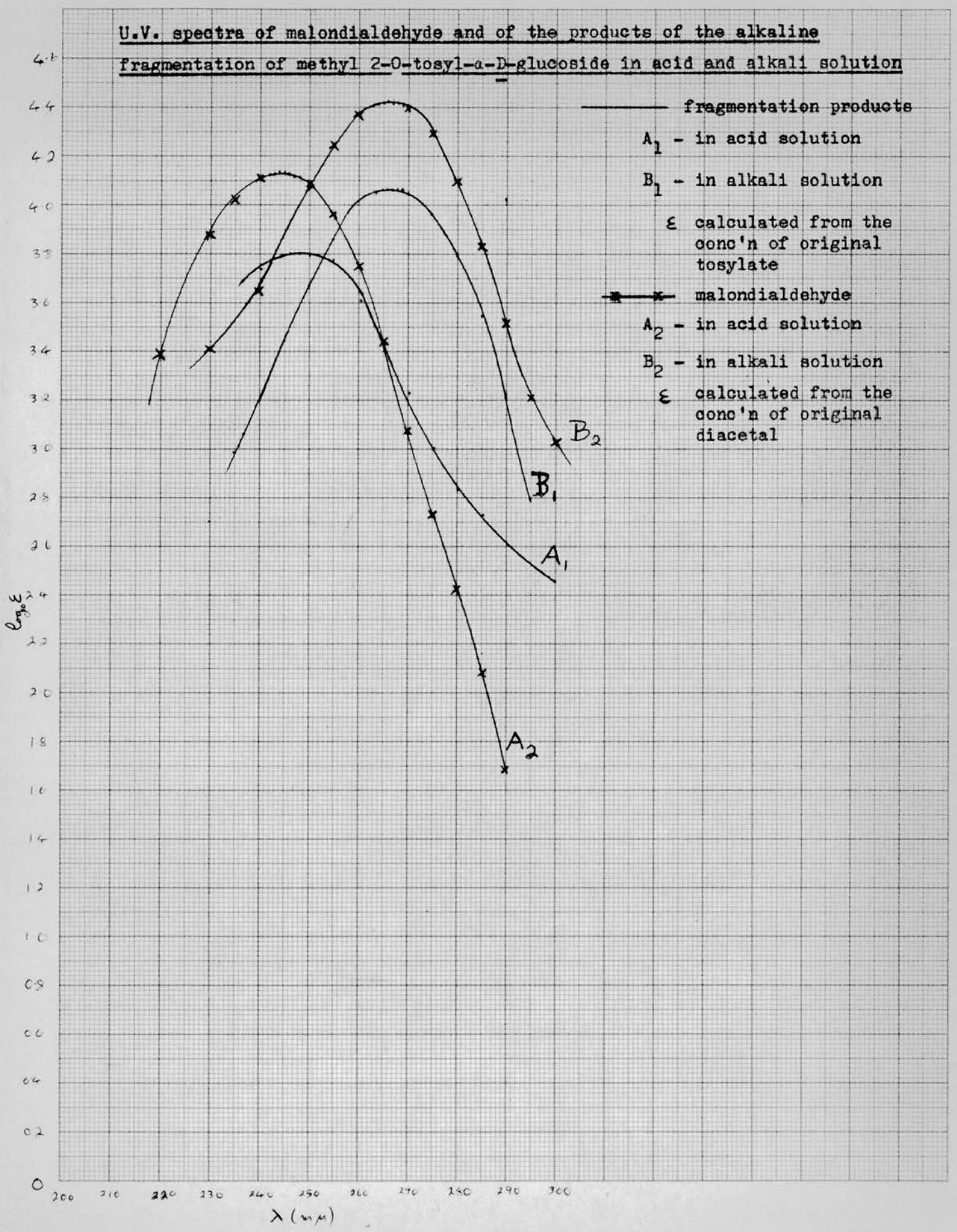
Column Chromatography. [90, 45]

Adsorption chromatography on chromatographic grade silica gel and alumina was employed, the silica being activated by oven-heating to 150° for 18 hours, the alumina being acid-washed, and of activity Grade V as defined by Brockmann.[91] Columns were generally packed in a slurry, and solvent systems were chosen empirically, the approximate non-polar ⟶ polar sequence, as recommended by Jeanloz,[90] being adhered to. Loadings of about 1 - 2% and 3 - 5% of the dry weights of silica gel and alumina respectively were used.

Using activated silica gel, and 1:1 benzene:ethyl acetate as development solvent, a clean separation of a 50:50 w/w mixture of 2,4-dinitropheny pyrazole and glyceraldehyde 2,4-dinitrophenylhydrazone was obtained. No resolution of the components

of the above-mentioned products of Brady's reagent with the alkaline fragmentation products occurred using a similar system. Using alumina, however, better results were obtained for this mixture. Elution of a column containing 50 mg. of the mixture with 1:1 benzene:ethyl acetate gave a pale orange crystalline solid (5 mg.) m.p. 200- 215°. 2% methanol/ethyl acetate eluted two further bands, each containing a few milligrams of coloured materials. Further elution with pure methanol gave a brick-red crystalline material (20 mg.) which, on crystallisation from ethyl acetate, melted at 205 - 208°. From U.V. spectroscopic studies (p.105), a mono-2,4-dinitrophenylhydrazone of malondialdehyde seemed a plausible structure for this final component, and analysis for carbon and hydrogen gave the following results: C, 44.2; H, 3.5. $C_9H_8N_4O_5$ requires C, 48.2; H, 3.6%.

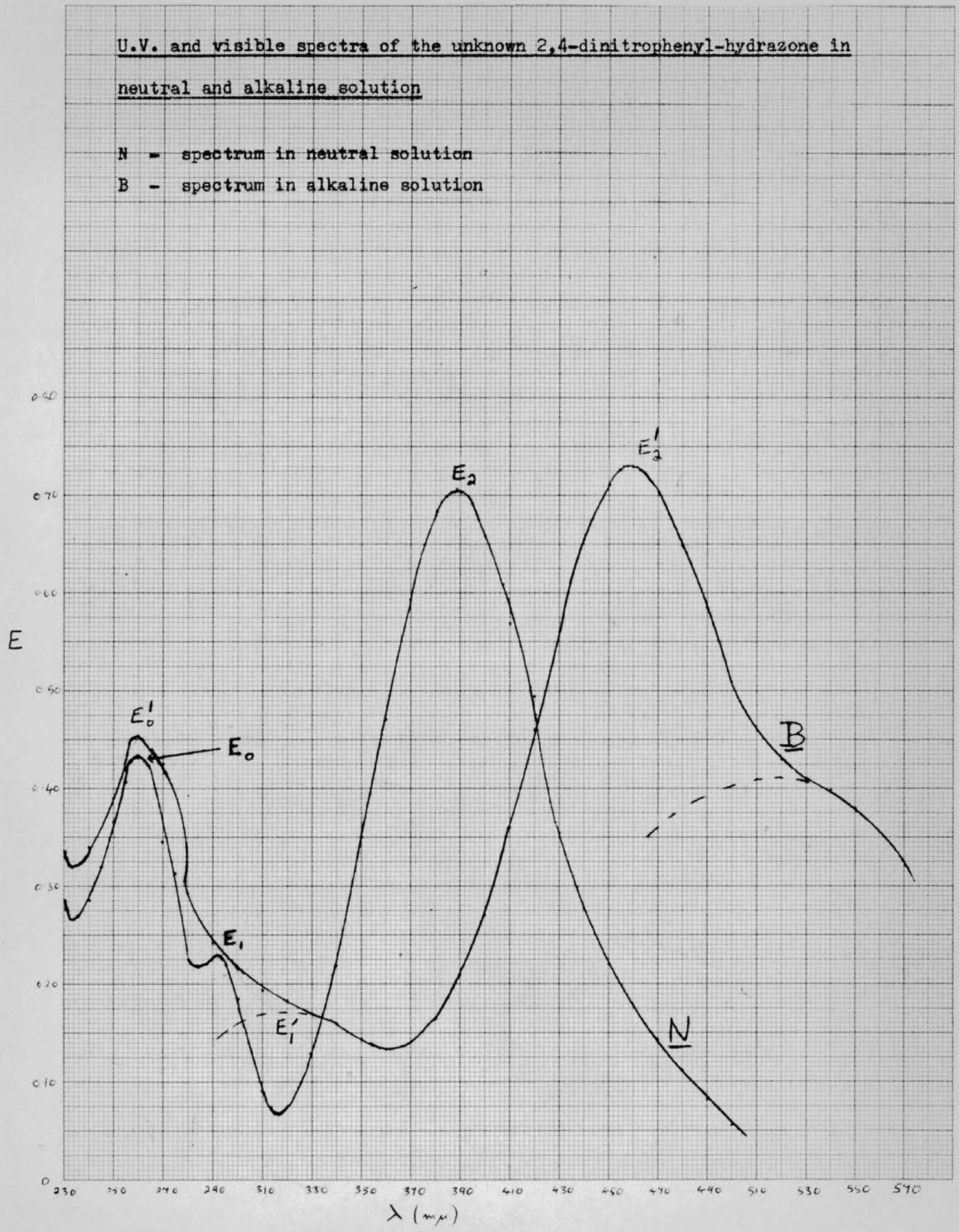
Spectroscopic Work. Kinetic details are not reported here, the results being largely irrelevant to this analytical study. The spectra of malondialdehyde, and of the fragmentation products and of the red material obtained therefrom by reaction with Brady's reagent followed by alumina chromatography, were measured on a Unicam S.P. 500 spectrophotometer. These spectra are reported graphically on pages 104 and 105.


U.V. spectra of malondialdehyde and of the products of the alkaline fragmentation of methyl 2-O-tosyl-α-D-glucoside in acid and alkali solution
fragmentation products
A1 - in acid solution
B1 - in alkali solution
ε calculated from the conc'n of original tosylate
malondialdehyde
A2 - in acid solution
B2 - in alkali solution
ε calculated from the conc'n of original diacetal
B2
B1
A1
A2
log ε
λ (mμ)

U.V. and visible spectra of the unknown 2,4-dinitrophenyl-hydrazone in neutral and alkaline solution

N - spectrum in neutral solution

B - spectrum in alkaline solution



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